STUDY OF PHYTOCHEMICAL CONSTITUENTS AND PHARMACOLOGICAL ACTIVITY OF LEAF EXTRACTS OF ANDROGRAPHIS ECHIOIDES-L-NEES

Dissertation Submitted in partial fulfillment of the requirement for the award of the degree of

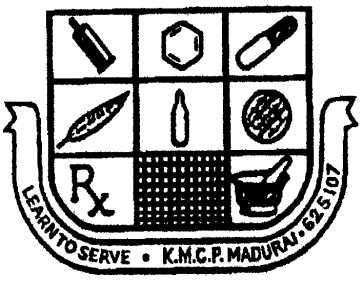
MASTER OF PHARMACY

IN

PHARMACEUTICAL CHEMISTRY

of

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY
CHENNAI



DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

K.M.COLLEGE OF PHARMACY
UTHANGUDI, MADURAI - 625 107

APRIL – 2012

CERTIFICATE

This is to certify that the dissertation entitled **"STUDY OF PHYTOCHEMICAL CONSTITUENTS AND PHARMACOLOGICAL ACTIVITY OF LEAF EXTRACTS OF ANDROGRAPHIS ECHIOIDES-L-NEES"** submitted by **Mr.M.RAJARAMAN** to The Tamilnadu Dr.M.G.R.Medical University, Chennai, in partial fulfillment of the requirement for the award of **Master of Pharmacy** in Pharmaceutical chemistry at K.M. College of Pharmacy, Madurai. It is a bonafide work carried out by him under my guidance and supervision during the academic year 2011-2012.

GUIDE

Mr.J.Raamamurthy, M.Pharm
Professor,
Dept. of Pharmaceutical chemistry,
K.M. College of pharmacy,
Uthangudi, Madurai-625107,
Tamilnadu.

H.O.D

Dr.S.Venkataraman, M.Pharm, Ph.D,
Professor and Head,
Dept. of Pharmaceutical chemistry,
K.M.College of Pharmacy,
Uthangudi, Madurai-625107,
Tamilnadu.

PRINCIPAL

Dr.S.Jayaprakash, M.Pharm, Ph.D.,
Professor and Head,
Dept. of Pharmaceutics,
K.M.College of Pharmacy,
Uthangudi, Madurai-625107,
Tamilnadu.

ACKNOWLEDGEMENT

Know god, Know peace

"The Teachers Gives Not Of Their Wisdom, But Rather Of Their Faith and Love"

It is my extreme elation to be honor and I have been blessed by lord to work under the guidance of ***Mr. J. Raamamurthy,*** *M.Pharm, Professor, Department of Pharmaceutical chemistry, K.M College of Pharmacy, Madurai, for his inspiration, encouragement, and constant suggestions which endless helped me to complete this project work successfully.*

It is my pleasant duty to express my deep sense of gratitude and heartfelt thanks to ***Prof. Mr. M. Nagarajan*** *M.Pharm, M.B.A, DMS (IM) DMS (BM),, Chairman, K. M College of Pharmacy, Madurai, for his inspiration and blesses on me.*

I express my honorable thanks to ***Dr. S. Jayaprakash,*** *M.Pharm., Ph.D., Principal and Head, Dept. of pharmaceutics, K.M College of Pharmacy, Madurai, for his encouragement extended throughout this work.*

I express my deep sense of gratitude and honor to ***Dr .S. Venkataraman,*** *M.Pharm., Ph.D., Vice Principal and Head, Department of Pharmaceutical chemistry, and* ***Mr. P. Muthumani,*** *M.Pharm, and* ***Mrs. R. Meera,*** *M.Pharm, Assistant Professors, Department of Pharmaceutical Chemistry, for their valuable suggestions.*

"Everyone everything around us is teacher"

I would like to express my profound sense of gratitude to ***Dr.N.Chidambaranathan,*** *M.Pharm., Ph.D., Head of the Department of Pharmacology, K.M. College of Pharmacy, for his valuable suggestions and encouragement in carrying out the experimental part of this work. Once again a Special thanks to him for his support.*

I wish to express a special sense of gratitude to ***Mr.M.S.Prakash,*** *M.Pharm, Professor, and* ***Mr.M.Boopathy,*** *M.Pharm, Department of Pharmaceutical Analysis, K.M. College of Pharmacy, for their valuable suggestions, guidance and help during analytical part of my work.*

It is my duty to say a special word of thanks to ***Mrs.M.Shanthi*** *B.A., M.L.I.Sc.M.Phil., Librarian and* ***Mrs.Anjelo Merina Priya*** *Library assistant.* ***Mr.C.Karthikeyan*** *MCA., Computer Lab Technician, K.M. College of Pharmacy for their timely help during this work.*

I wish to express a special thanks to ***Mrs. S. Shanmugapriya****, D.Pharm,* ***Ms. A. Vijaya lakshmi,*** *B.Sc, Lab Technicians, for their kind and timely help during lab work.*

"Family is heaven in a heartless world"

I humbly submit this work to the ***Lord Almighty****, who let me best forever.*

I wish to express my deepest love and thanks to my beloved ***Uncle Mr.N.C.Ramalingam, my Parents****,* ***sisters and their children*** *who think and dedicate themselves always for my future.*

I owe a great many thanks to a great many people who helped, supported and encouraged me during the writing of this book.

Friendship: One soul inhabiting many bodies

Friends: kisses blown by angels

I wish to convey my gratitude of thanks to my friendly brother ***Mr.M.Jegadheeswaran,*** *and dear classmates* ***Mr.Anup.P, Mr.Juno Raphael, Mrs.Swathy lakshmi,*** *and* ***Ms.Vidya*** *who shared their precious time, valuable suggestions and innovative ideas with me to complete my thesis in a successful manner.*

I like to express great thanks to my batchmates ***Ms.M.Princy prasanna, Mr.G.Somavel, Ms.Kinnera, Mr.Kirankumar, Mr.Ravikishore, Ms.Sravani, Mr.Malligarjuna, Mr.P.Natraj, Mr.shakthivel, Mr.Sivaraj, Mr.Jeeva, Mr. Mahesh, Mr.Selvaganesh, Mr.Balakumar, Mr.Logu Mr.Shahul and Mr.Sudhakar*** *who gave me great support and encouragement throughout my project.*

Languages are insufficient to thank my topmost friends ***Mr.K.Jothibasu, Mr.P.Arunkumar, Mr.D.Azhagumani, Mr.K.Cholaraja, Mr.S.Sudhakar, Mr.B.Venkatesh, Mr.R.Karthick, Mr.V.Satheesh, Mr.D.Dinesh*** *and* ***Mr.N.Rajeshkumar*** *who helped directly and indirectly to complete my work.*

It is my extreme pride to convey my gratitude to my closest friends ***Mr.P.Suresh, Mr.K.Nagapandy, Mr.K.Ranjithkumar and Mr.M.Balamurugan*** *who shown their possible guidance to complete my project in a smart way.*

I wish to convey my gratitude of thanks to ***laser point*** *for their high quality printing and binding.*

I might have forgotten to name few people behind this work, but still I really thank to all concerned individuals for their support to complete this work successfully in time.

THANKS TO ALL

CONTENTS

INTRODUCTION [1-8]

During the past few years, due to the view of all aspects of ecology, these have been renewed focus with the interest in so called natural foods and drugs.

The availability of extremely wide range of these natural products ranging from fenugreek tea to ginseng chewing gum has stimulated the public to learn more about these natural products as medicine.

Nature has provided a complete **"Store house"** of remedies to rectify all illness of mankind. It provides us a wide range of medicines in the forms of herb, plant, etc to eradicate many diseases without causing much of toxic effects. Plant kingdom holds many species of plants containing pharmacologically active constituents.

SOME BASIC TERMS;

Botany is a branch of biology which explains the study of plant life, including structure, growth, taxonomy, systematic, reproduction, metabolism, physiology, biochemistry, development, diseases, ecology, and evolution of plants.

Ethno Botany is the study of the relationship between plants and people and their culture.

Phytochemistry can be defined as the study of phytochemical produced in plants, it also describing the isolation, purification, identification, and structure of the large number of secondary metabolic compounds found in plants.

An herb, in botany, is a plant that does not form a woody stem, and in particular climate usually dies, either completely (annual herb) or back to the roots (perennial herb) by the end of the growing season. **Examples** for perennial herbs include bulbs, Peonies, Hosta, grasses and Banana.

A medicinal herb is different from botanic term "herb". It refers to any part of the plants used for medicinal purposes. A medicinal herb can be a real herbal plant, a shrub, other woody plant, or a fungus. The used part may be the seeds, berries, leaves, barks, roots, fruits, or other parts of a plants, or mushroom, which may be considered "herbs" in medicinal or spiritual use.

HISTORY OF MEDICINAL PLANTS:

The role of plants in the treatment of disease can be explained by their employment in all major system of medicine.

In India some of the ancient records like **"Rig-Veda samhita"** have explained about the use of medicinal plants as a remedy for many diseases.

Some other literatures such as **Ayurveda** and **Unani** system of medicines also deal about the use of herbal drugs as major part of chemotherapy.

Sanskrit literature like **Raghuvamsa** written by Kalidas consist more information about morphological features of many plants.

Even today in this indigenous system of medicine, the plant extract are used as effective remedy against various diseases.

Globally the medicinal plants were used as a **remedy for many illnesses**. These can be proved by many literatures and records which have been written.

- ♣ Babylonians made clay models of the human body and they have written some records which gives the proof for that they were aware of medicinal effects of many medicinal plants.

- ♣ Egyptians were well known about the human anatomy as well as knowledge of the medicinal uses of many plants. These have been recorded in **Papyrus Ebers** written in 1550 BC. This document is now preserved at the University of Leipzig.

- ♣ A Greek physician, Dioscorides wrote his **De Materia Medica** in 78 A.D in which he described about 600 plants that were known to have medicinal properties.

- ♣ Galen, a Greek pharmacist-physician has described method of preparing plant drug from the plant origin. He has written more than 20 books because of his reference the term **Galenical Pharmacy** was originated.

ROLE OF MEDICINAL PLANTS;

- ♣ Medicinal plants have played an essential role in the development of human culture. For Example, religions and different ceremonies.
- ♣ Many of the modern medicines are produced indirectly from medicinal plants, for example aspirin.
- ♣ Plants are directly used as medicines by a majority of cultures around the world, for example Chinese medicine and Indian medicine.
- ♣ Many food crops have medicinal effects, for example garlic.
- ♣ Medicinal plants are resources of new drugs. It is estimated there are more than 250, 000 flower plant species.
- ♣ Studying medicinal plants helps to understand plant toxicity and protect human and animals from natural poisons.
- ♣ Cultivation and preservation of medicinal plants protect biological diversity, for example metabolic engineering of plants.

CLASSIFICATION OF DRUGS

In Pharmacognosy drugs are classified as follows.

Morphological classification

In this classification, drugs are grouped according to the part of the plant or animal such as roots, leaves, organs glands.

Taxonomical classification

In this class, drugs are classified according to their natural relationship or phylogeny among plants. Example, cremocorp fruits are considered with other members of umbelliferae.

Pharmacological or therapeutical classification

This class explains the drugs according to their pharmacological action or therapeutic application of the drug. Example, cascara, sagrada, senna, podophyllum are called as cathartics.

Chemical classification

In this, drugs are classified according to their chemical constituents which are responsible for action. Example mydriatic alkaloids (atropine, scopolamine) characterize the solanaceae.

BIOCHEMISTRY OF PLANTS

Biosynthetic pathways;

- ♥ Pentose pathway
- ♥ Glycolysis
- ♥ TCA cycle

Primary metabolites of plants;

- ✓ Carbohydrates
- ✓ Proteins
- ✓ Fatty acids
- ✓ Isoprene
- ✓ Squalene

Secondary metabolites of plants;

- ✓ Glycosides, gums and mucilage, streptomycin
- ✓ Phenols, tannins, lignin, alkaloids, peptides
- ✓ Fats and waxes, anthroquinones
- ✓ Terpenes
- ✓ Steroids

EVALUATION OF NATURAL PRODUCTS

Evaluation of any drugs deals with determination of the identity, purity and quality of the drug.

Identity:

Identity deals with following characters

Morphological characters: In this the identity of the drug can be determined by their morphological characters and characteristics of each group as leaves, barks, and fruits etc. e.g. Seeds of Strophanthus, Caraway and Dill are distinguished by their morphology.

Sensory characters: In this the plant drugs are identified by sensory characters such as color, smell, taste and consistency e.g. leaves of lobelia should be in green color. If it shaded it becomes pale color.

Microscopic characters: In this drugs are identified by microscopic characters such as type of stomata, trichomes, and calcium oxalate crystals and also by observing transverse and longitudinal sections e.g. barks of cinchona consists phloem fibres.

Physical characters: In this drugs are identified by observing certain physical constant such as solubility, specific gravity, viscosity, refractive index etc.

Purity:

To standardize natural products, the presences of foreign organic and inorganic matters have to be evaluated.

Foreign organic matters: These may be due to the presence of other parts of the same plants or other plants. These organic matters should be removed manually or some other method like sedimentation method in which drug is boiled with chloroform to settle down the organic matters.

Foreign inorganic matters: These may be due to the presence of inorganic metals and some other ash and sulphated ash and moisture. Moisture content can be determined by Karl Fischer method. These impurities also be determined by crude fiber and fluorescence analysis.

Quality:

Various methods are used to check the quality of the natural products

Physical methods: These include checking of swelling factor in mucilage containing drugs, viscosity in gums, and froath number in saponins containing drugs and congealing point in anithole containing drugs and also include determination of solubility of extracts.

Chemical methods: These include qualitative analysis of natural products can be done by gravimetric, volumetric, colorimetric and fluorimetric methods. E.g. Total alkaloids in solanaceous drugs can be determined by chemical assays, Vitamin A can be determined by antimony trichloride, Ergot alkaloids can be determined by para dimethyl amino benzaldehyde.

ANALYTICAL METHODS FOR EVALUATION OF PHYTO CHEMICALS:

Spectroscopic methods [50]:

UV-Visible spectroscopy:

This spectroscopy method is helpful to determine the conjugation of the phyto chemicals which are obtained from the plant sources.

IR Spectroscopy:

This method used to elucidate the functional groups of the phyto chemicals. The IR spectrum of a compound having two regions namely functional group region and finger print region will provide sufficient information about the structure of the compound. The peaks present in the spectrum depend upon the respective functional groups.

Principle: Changes in the vibrational and rotational levels

Method: Samples are prepared by using KBr pellet in which KBr and sample were mixed by means of triturating both in the ratio of 100: 1 respectively. Then this sample kept in holder and irradiated by IR beams to get a spectrum of compound.

^{1}H NMR Spectroscopy:

This spectroscopic method is used to find out the nature and arrangement of protons present in the phyto chemical compounds. In this method phytochemical samples are kept in magnetic field then irradiated with electromagnetic radiation. This will give the NMR spectrum which having the characteristic signals according to their chemical shift values of the protons.

^{13}C NMR Spectroscopy:

This will provide complete information about the carbon number of the phyto chemical compounds.

Mass Spectroscopy:

This will provide the molecular mass of the compound.

Chromatographic methods [47]:

Chromatography is a group of techniques for the separation of the compounds of mixtures by their continuous distribution between two phases one of which is moving past the other.

Various chromatographic methods are used in the identification, Isolation and Purification of phyto chemicals. They are,

- ♣ Paper chromatography
- ♣ Thin layer chromatography
- ♣ High performance thin layer chromatography
- ♣ Column chromatography
- ♣ High performance liquid chromatography
- ♣ Gas chromatography
- ♣ Ion exchange chromatography

Hyphenated Techniques:

These techniques are implies both chromatographic as well as spectroscopic methods. They are,

- ♣ LC-MS (Liquid chromatography - Mass spectroscopy)
- ♣ GC-MS (Gas chromatography – Mass spectroscopy)

REVIEW OF LITERATURE [9-41]

PHYTOCHEMICAL STUDIES

* **B.Jayaprakasam et al. [1999]** have done the reinvestigation of the whole plant of *Andrographis echioides* has led to the isolation of a new flavone, dihydroechioidinin together with four known flavones, echioidinin, echioidin, skullcapflavone I 2'-O-methyl ether, and skullcapflavone I 2'-O- glucoside. The structure of dihydroechioidinin was established as (2S)-5, 2'-dihydroxy-7-methoxyflavanone on the basis of spectral and chemical evidence.

* **T. R. Govindachari et al. [1965]** have isolated echioidinin, a new flavone and echioidin its glucoside from *Andrographis echioides*, and reported. On the basis of spectral, degradative and synthetic evidence, echioidinin is shown to be 5, 2'dihydroxy-7-methoxyflavone.

* **T. R. Govindachari et al. [1965]** have isolated echioidin, the new flavones glucoside from *Andrographis echioides* on the basis of degradative and spectral evidence and synthesis, is shown to be 5-hydroxy-2'- β-D-glucosidoxy-7-methoxyflavone (echioidinin-2'-β-D-glucoside).

* **Y.Koteswara rao et al. [2004]** have isolated two flavonoids, identified as 5,7,2',3'-tetramethoxyflavanone and 5-hydroxy 7, 2',3'trimethoxyflavone, as well as several other flavonoids, andrographolide diterpenoids and polyphenols, were obtained from the phytochemical investigation of the whole plant of *Andrographis paniculata* which is a well known medicinal plant. The structures of these compounds were established with the aid of spectroscopic methods, including analysis by 2D NMR spectroscopy.

* **Poonam kulyal et al. [2010]** have done the phytochemical investigation of the aerial parts of *Andrographis paniculata*, gives diterpenic constituents andrographolide, 14-deoxy-11,12-didehydroandrographolide, 14-

deoxyandrographolide, 3,14-dideoxyandrographolide, 14-deoxy-11-oxoandrographolide, 14-deoxy-12-hydroxy andrographolide, neoandrographolide, andrographiside and 14-deoxyandrographiside. The structures of these compounds have been established on the basis of spectral data analysis.

* **Muntha K. Reddy et al. [2003]** have done phytochemical investigation of the roots and aerial parts of *Andrographis paniculata* Nees yielded a new flavone, 5-hydroxy 7,2',6'-trimethoxyflavone and an unusual 23-carbon terpenoid, 14-deoxy-15-isopropylidene-11,12-didehydroandrographolide together with five known flavonoids and four known diterpenoids. The structures of these compounds were determined on the basis of spectral and chemical studies.

* **Guo-Cai Wang et al. [2009]** have isolated Andrographolactone, possessing an unprecedented diterpene skeleton, from the ethyl acetate extract of the aerial parts of *Andrographis paniculata.* Its structure was established by NMR, IR, UV, and HRESIMS data and confirmed by X-ray diffraction analysis. A possible biogenetic pathway of Andrographolactone was also proposed. Bioassay showed that Andrographolactone exhibited cytotoxic activity.

* **P. Hari Kishore et al [2003]** have isolated three flavonoids, 5,7,2',3',4'-pentamethoxyflavone, 2'-hydroxy-2,4',6'-trimethoxychalcone and dihydroskullcapflavone I, together with 17,19,20-trihydroxy-5β, 8α H, 9β H,10α-labd-13-en-16,15-olactone, a known diterpenoid and six known flavonoids, 5-hydroxy-7,8-dimethoxyflavanone, 5-hydroxy-7,8,2',3',4' pentamethoxyflavone, 5,2'-dihydroxy-7-methoxyflavanone, 5,2'-dihydroxy-7,8-dimethoxyflavone, 5,2'-dihydroxy-7-methoxyflavone and 5,2'-dihydroxy-7-methoxyflavone 2'-O-β-D-glucopyranoside from the whole plant of *Andrographis lineata*. The structures of these compounds were elucidated on the basis of spectral and chemical studies.

* **Y. Koteswara Rao et al. [2002]** have isolated two new 2'-oxygenatedflavones, 5,7,2'-trimethoxyflavone and5,7,2',4',6'-pentamethoxyflavone from the whole

plant of *Andrographis viscosula* along with three known flavones, echioidinin, 5,2',6'-trihydroxy-7-methoxyflavone, and echioidin. The structures of these compounds were elucidated on the basis of 1D and 2D NMR spectral studies.

* **P. K. Singh et al. [2009]** have done complete normal coordinate analysis for neoandrographolide in terms of the calculation by using Wilson's G-F matrix method and Urey Bradley force field. *Andrographis paniculata* has been reported for its potent hepatoprotective. Andrographis paniculata has been reported to have antisecretory (antidiarrhoeal), immunostimulant, antimalarial, antifilarial activity. It is also reported to have anticancer, anti HIV, antiinflammatory, hypotensive action. In addition, it has found to be effective in myocardial infarction.

* **Lixia Chen et al. [2007]** have isolated two pairs of ent-labdane diterpenoid lactones stereo isomers (1-4) including three new compounds (1-3) from the 85% ethanol extract of the aerial parts of Andrographis paniculata. The structures of these compounds were identified as 7R-hydroxy-14-deoxyandrographolide (1), 7S-hydroxy-14-deoxyandrographolide (2), 12S,13S- hydroxyandrographolide (3), and 12R,13R-hydroxyandrographolide (4) by spectroscopic data analyses and calculated 13C NMR data at the B3LYP/6-311++G(2d,p)//B3LYP/6-31G* level using the GIAO method. The 12S-configuration of 4 was revised to 12R based on the spectroscopic data. The antiproliferative activities of the two pairs of stereo isomers and 14 other ent-labdane diterpenoid derivatives were determined in human leukaemia HL-60 cells. Andrographolide (7) and isoandrographolide (12) exhibited higher antiproliferative activities than other ent-labdane diterpenoids with GI50's of 9.33 and 6.30 μM, respectively.

PHARMACOLOGICAL STUDIES

* **Premkumar et al. [2010]** have studied the antioxidant potential of ethanolic extract of *Andrographis echioides* and *Boerhavia diffusa* which was evaluated by determining the levels of enzymatic and non-enzymatic antioxidants. Their results showed that both the plant extracts possessed significant levels of enzymatic and non-enzymatic antioxidants. However, *Andrographis echioides* showed higher levels of enzymatic and non-enzymatic antioxidants than *Boerhavia diffusa*.

* **Radha. R et al. [2011]** have evaluated the preliminary phytochemical and antimicrobial activity of the successive extracts (Petroleum ether, Chloroform, Acetone and Methanol) of the leaves and stems of *Andrographis echioides* against two Gram-positive, two Gram negative bacteria and two fungi using disc diffusion method. The results revealed the presence of alkaloids, flavonoids, glycosides, steroids, phenols, tannins and saponins in leaf and stem. Highest activity was observed in Gram-positive bacteria in leaf and stem extracts. The fungal activity was showed highest against *Candida albicans*.

* **S.K. Basu et al. [2009]** have investigated to study the anti-inflammatory, analgesic and anti-pyretic properties of total extract and three fractions (ether, chloroform, and ethyl acetate) from *Andrographis echioides* (Acanthaceae) in rats and mice. Dose of 200 to 400 mg kg^{-1} of each extracts were used in carrageenan induced paw oedema, cotton-pellet granuloma in rats, writhing nociception in mice and yeast induced hyperpyrexia in rats. All compounds reduced paw oedema in comparison to the control group at 5h post carrageenan injection. The total ether and ethyl acetate extracts were similar to phenlbutazone ($p<0.001$), while chloroform extract was weaker than phenylbutazone in reduction of paw oedema and cotton-pellet granuloma. All extracts as well as paracetamol induced antinociception in writhing in comparison to control. Positive results for flavonoids and phenolic compounds were investigated by phytochemical analysis. The activities might be due the presence of flavonoids and phenolic compounds.

These data showed that different extracts of Andrographis echioides produce antinociceptive, anti-inflammatory, analgesic and anti-pyretic activities.

* **K. Kavitha et al. [2009]** have investigated the methanolic extract of *Andrographis echioides* for its hepatoprotective and antioxidant effects against acetaminophen induced Hepatotoxicity in Wistar albino rats. The plant extract (200 and 400 mg kg^{-1}, p.o/day for 10 days) showed a remarkable hepatoprotective and antioxidant activity. Hepatotoxicity was induced by acetaminophen at the dose of 750 mg kg p.o for 10 days. The serum marker enzymes such as aspartate amino transferase (AST), alanaine amino transfease (ALT), alkaline phosphates (ALP), total bilirubin and gamma glutamate transpeptidase (GGTP), lipid peroxidise (LPO), were significantly increased with a reduction of liver total protein, superoxide dismutase (SOD), catalase, glutathione peroxidise (GPx), and glutathione-S-transferase (GST), in acetaminophen induced rats. The activity of these extracts was comparable to the standard drug, silymarin (50 mg kg^{-1}, p.o). Treatment with different doses of aerial parts of methanolic extract of Andrographis echioides produced only mild degenerative changes and absence of centrilobular necrosis indicating its hepatoprotective efficiency.

* **M. Sermakani et al. [2011]** have designed with the objective to examine the petroleum ether, acetone, chloroform and methanol extracts of *Andrographis paniculata* leaves and stems, in order to evaluate the chemical composition, investigate it's *in vitro* antimicrobial potential against strains of *Enterococcus faecalis, Streptococcus pyogenes, Klebsiella pneumonia*, *Proteus vulgaris*, *Candida albicans and Aspergillus flavus*. Phytochemical analysis revealed the presence of flavonoids, alkaloids, glycosides, steroids, phenols, tannins and saponins. The antibacterial activity is more significant against Gram positive bacterium *Enterococcus faecalis* whereas the antifungal activity is more significant against *Aspergillus flavus.* These results may justify the popular use of this species as it has antimicrobial activity. However, in order to evaluate possible

clinical application in therapy of infectious diseases, further clinical trials are required.

* **S.K. Ojha et al. [2009]** have found that, Hydroalcoholic extract of *Andrographis paniculata* prevented isoproterenol induced increase in lipid peroxidation and increased the activities of antioxidant enzymes viz. super oxide dismutase, catalase, glutathione peroxidase and the levels of reduced glutathione in hearts. In addition, the extract also prevented the leakage of lactate dehydrogenase from heart and salvages the heart from isoproterenol induced myocardial ischemic injury. The results indicate the antioxidant, antilipid peroxidative and antiischemic activity of *Andrographis paniculata* and justify its use in ischemic heart diseases.

* **Abubakar Sule et al. [2011]** have evaluated Non-polar (dichloromethane) and polar (MeOH and aqueous) extracts of Andrographis paniculata (whole plant) for in vitro antibacterial activity against 10 skin disease causing bacterial strains (6 gram positive strains; Staphylococcus saprophyticus, Staphylococcus epidermis, Staphylococcus aureus, Streptococcus pyogenes, Bacillus anthracis, Micrococcus luteus) and 4 gram negative strains (Proteus mirabilis, Proteus vulgaris, Neisseria meningitis, Pseudomonas aeruginosa) using disc diffusion method at three different concentrations; 1000, 500 and 250 µg/disc respectively. The extracts showed significant antibacterial activities against both Gram-positive and Gram-negative bacterial strains tested. Highest significant antibacterial activity was exerted by the aqueous extract against M. luteus at 1000 µg/disc and the least activity was exhibited by the DCM extract against N. meningitis at 250 µg/disc. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) observed were between 150 to 300 µg/ml and 250 to 400 µg/ml respectively, depending on microorganism and the nature of various extracts. Time-kill experiments indicated that A. paniculata extracts have bactericidal characteristic against most of the Gram positive bacteria and bacteriostatic activity against both Gram negative and Gram positive bacteria.

These results candidly suggest the presence of promising antibacterial substances in the polar as well as non-polar extracts which could be the source of potential phytomedicine for the treatment of skin infections caused by the pathogenic bacterial strains. Our findings explicitly support its traditional claims and form a strong basis for further sincere efforts to explore Andrographis paniculata's antibacterial potential to treat skin frailties efficaciously.

* **S. Meenatchisundaram et al. [2009]** have tested Methanolic extracts of Andrographis paniculata and Aristolochia indica plants for antivenom activity against Daboia russelli venom. Both plant extracts effectively neutralized the D. russelli venom induced lethal activity. About 0.15 mg of A. paniculata and 0.14 mg of A. indica plant extracts were able to completely neutralize the lethal activity of 2LD50 of D. russelli venom. Various pharmacological activities including oedema, haemorrhagic, coagulant, fibrinolytic and phospholipase activities were studied and these pharmacological activities were significantly neutralized by both the plant extracts. The above observations confirmed that A. paniculata and A. indica plant extracts possess potent snake venom neutralizing capacity and could potentially be used for therapeutic purposes in case of snakebite envenomation.

* **Piengpen Thisoda et al. [2006]** had investigated the three active diterpenoids from the plant, *Andrographis paniculata* including aqueous plant extracts, for the inhibitory effect on platelet aggregation in vitro. The results indicated that andrographolide (AP1) and 14-deoxy-11,12-didehydroandrographolide (AP3) significantly inhibited thrombin-induced platelet aggregation in a concentration- (1–100 μM) and time-dependent manner while neoandrographolide (AP4) had little or no activity. AP3 exhibited higher antiplatelet activity than AP1 with IC50 values ranging from 10 to 50 μM. The inhibitory mechanism of AP1 and AP3 on platelet aggregation was also evaluated and the results indicated that the inhibition of extracellular signal-regulated kinase1/2 (ERK1/2) pathway may contribute to antiplatelet activity of these two compounds. In addition, standardized aqueous

extracts of A. paniculata containing different amounts of AP3 inhibited thrombin-induced aggregation to different degrees. The extracts significantly decreased platelet aggregation in a concentration-(10–100 μg/ml) and time-dependent manner. However, the extract with high level of AP3 (Extract B) (IC50 values=50–75 μg/ml) showed less inhibitory activity against thrombin than the extract with lower level of AP3 (Extract A) (IC50 values=25–50 μg/ml). These results indicate that the standardized A. Paniculata extract may contain other antiplatelet compounds rather than AP1 and AP3, which contribute to high antiplatelet activity. Therefore, the consumption of A. paniculata products may help to prevent or treat some cardiovascular disorders i.e. thrombosis; however, it should be used with caution by patients with bleeding disorders.

* **Rammohan Subramanian et al. [2008]** have evaluated *Andrographis paniculata's* ethanolic extract to screen the effect on insulin resistance using a combination of fat-fed diet and low dose streptozotocin. The glucose-insulin index as a measure of insulin action on glucose disposal rate was calculated during the intraperitoneal glucose tolerance test. Oral administration of 1000 mg/kg extract to rats was able to cause a significant ($p<0.05$) reduction of elevated glucose-insulin index, signifying a potential insulin sensitizing effect. Oral administration of the extract at a dose of 1000 mg/kg once daily for 30 days to streptozotocin-diabetic rats increased the hypoglycaemic responses to incremental dosing of exogenous insulin, thus causing an increase in insulin sensitivity. The results seem to suggest that oral administration of Andrographis paniculata ethanolic extract may have the ability to improve insulin sensitivity and delay the development of insulin resistance, and may thus have a role in amelioration of insulin resistance in patients. However, its potential use in humans can only be validated by thorough investigation.

* **Vetriselvan.S et al. [2011]** have studied the effect of *Andrographis paniculata* extract on CCl_4 induced hepatic damage in rats. The degree of protection was measured by physical, biochemical changes. Pretreatment with extract

significantly prevented the physical, biochemical changes 1 induced by CCl_4 in the liver. The effects of andrographis paniculata could be useful in preventing chemically induced2 acute liver injury. It can be concluded that the aqueous extract of Andrographis paniculata almost significant effective in the standard drug.

* **Sutha et al. [2010]** have studied the hepatoprotective effect of crude methanolic extracts of *Andrographis paniculata* on mice. The phytochemical screening of the crude methanolic extracts of Andrographis paniculata plant was also determined followed by the confirmation of the active compound using Thin Layer Chromatography. The hepatoprotective activity of methanolic extracts of Andrographis paniculata was evaluated against paracetamol induced (500 mg/kg) hepatic damage in mice. The extracts at doses of 10 mg/kg and 100 mg/kg were orally administered at 24 and 72 hours time interval in each group. Histological analysis of the liver and the liver protein content was determined. The results of the study indicated that the crude extracts of Andrographis paniculata at both doses exhibited a significant protective effect in the liver morphology of the paracetamol induced hepatotoxicity in mice. There was also a significant decrease ($P<0.05$) the liver protein content of the hepatotoxic mice after the treatments. Thin Layer Chromatography confirmed the presence of active compound, diterpene lactone or andrographolide which has contributed to the hepatoprotective activity of Andrographis paniculata. Hence, the results of the present study indicated that Andrographis paniculata possess hepatoprotective effects which could compromise the medicinal use of this plant in folk medicine.

* **Kanokwan Jarukamjorn et al. [2008]** have reviewed on pharmacological aspects of *Andrographis paniculata* on health and its major diterpenoid constituent andrographolide. The aim of this review is to compiling consequential compendium of pharmacological benefits of health on this plant and major diterpenoid constituent andrographolide that have been tested in various experimental models using modern scientific methodologies.

* **K. Sheeja et al. [2006]** had analyzed the antiangiogenic activity of *Andrographis paniculata extract* (APE) and its major component andrographolide (ANDLE) using both in vitro and in vivo models. Intraperitoneal administration of APE and ANDLE significantly inhibited the B16F-10 melanoma cell line induced capillary formation in C57BL/6 mice. Analysis of serum cytokine profile showed a drastic elevation in the proinflammatory cytokines such as IL-1β, IL-6, TNF-α and GM-CSF and the most potent angiogenic factor VEGF in angiogenesis induced animals. Treatment of APE and ANDLE significantly reduced this elevated levels. Moreover, VEGF mRNA level in B16F-10 cell line showed a reduced level of expression in the presence of APE and ANDLE. Serum NO level which was increased in B16F-10 melanoma injected control animals was also found to be significantly lowered by the administration of APE and ANDLE. Antiangiogenic factors such as TIMP-1 and IL-2 level was elevated in APE and ANDLE treated angiogenesis induced animals. In the rat aortic ring assay APE and ANDLE inhibited the micro vessel outgrowth at non toxic concentrations. Taken together our results demonstrate that APE and ANDLE inhibit the tumor specific angiogenesis by regulating the production of various pro and antiangiogenic factors such as proinflammatory cytokine, nitric oxide, VEGF, IL-2 and TIMP-1.

* **R. Ajaya Kumar et al. [2004]** have evaluated the anticancer and immunomodulatory activity of the methanolic extract of *Andrographis paniculata* in human cancer and immune cells. The methanolic extract of Andrographis paniculata was fractionated into dichloromethane, petroleum ether and aqueous extracts and screened for bioactivity. Their results indicated that the dichloromethane fraction of the methanolic extract retains the active compounds contributing for both the anticancer and immunostimulatory activity. Dichloromethane fraction significantly inhibits the proliferation of HT-29 (colon cancer) cells and augments the proliferation human peripheral blood lymphocytes (HPBLs) at low concentrations. On further fractionation of the dichloromethane extract we could isolate three diterpene compounds, i.e. andrographolide, 14-

deoxyandrographolide and 14-deoxy-11,12-didehydroandrographolide. Andrographolide showed anticancer activity on diverse cancer cells representing different types of human cancers. Whereas all the three molecules showed enhanced proliferation and interleukin-2 (IL-2) induction in HPBLs.

* **E. Amroyan et al. [1999]** have investigated Andrographolide, an active principle of the Chinese drug *Andrographis paniculata*, for its suggested influence on the biosynthesis of eicosanoids and the platelet-activating factor (PAF). Whereas in isolated human polymorph nuclear leukocytes (PMNL) no influence on the biosynthesis was found, it could be shown that andrographolide inhibits PAF-induced human blood platelet aggregation in a dose dependent manner (ICso 5 pM). These results indicate that andrographolide has a mechanism of action different from that of non-steroidal anti-inflammatory drugs (NSAID) and most likely associated with the cardiovascular and antithrombotic activity described of Andrographis paniculata.

* **Reddy VL et al. [2005]** have isolated 14-deoxy-11,12-didehydroandrographolide, andrograpanin, 14- deoxyandrographolide, (+/-)-5-hydroxy-7,8-dimethoxyflavanone, and 5-hydroxy- 7,8-dimethoxyflavone from the aerial parts of *Andrographis paniculata* and their structures were established by spectral data. All the isolates were tested for the anti-HIV and cytotoxic activity.

* **Wiart C et al. [2005]** have isolated Andrographolide, neoandrographolide and 14-deoxy-11,12- didehydroandrographolide, ent-labdene diterpenes from *Andrographis paniculata* showed viricidal activity against herpes simplex virus 1 (HSV-1). None of these compounds exhibited significant cytotoxicity at viricidal concentrations.

* **Singha PK et al. [2003]** have evaluated the antimicrobial activity of aqueous extract, andrographolides and arabinogalactan proteins from *Andrographis paniculata.* The aqueous extract showed significant antimicrobial activity, which

may be due to the combined effect of the isolated arabinogalactan proteins and andrographolides.

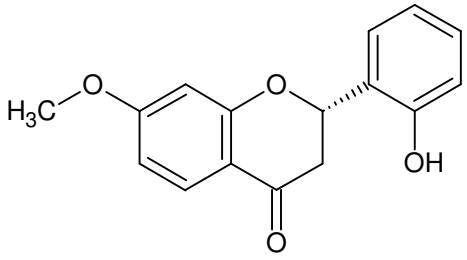
* **Zhang CY et al. [1996]** have studied the hypotensive activity of an aqueous extract of *Andrographis paniculata* using chronic intraperitoneal infusions by osmotic pumps. The extract exhibited a dose-dependent hypotensive effect on the systolic blood pressure (SBP) of spontaneously hypertensive rats (SHR). 2. The optimum hypotensive dose determined was repeated in a study in SHR and their normotensive controls, Wistar-Kyoto (WKY) rats, to demonstrate its comparative effects on the SBP, plasma and lung angiotensin-converting enzyme (ACE) activities, as well as on lipid peroxidation in the kidneys, as measured by the thiobarbituric acid (TBA) assay. 3. The extract significantly lowered the SBP of both SHR and WKY rats. 4. Plasma, but not lung, ACE activity and kidney TBA level were significantly lower in extract-treated SHR when compared with vehicle treated SHR controls 5. Plasma and lung ACE activities as well as kidney TBA levels were not significantly different between extract- and vehicle-treated WKY rats. 6. This study indicates that the aqueous extract of A. paniculata lowers SBP in the SHR possibly by reducing circulating ACE in the plasma as well as by reducing free radical levels in the kidneys. The mechanism(s) of hypotensive action seems to be different in WKY rats.

* **Borhanuddin M et al. [1994]** have tried hypoglycaemic effect of *Andrographis paniculata* in various ways. Water extract of AP 10 mg/kg body weight can prevent induction of hyperglycemia significantly ($P < 0.001$) induced by oral administration of glucose 2 mg/kg body weight. But any how failed to do so in adrenaline induced hyperglycemia. It also failed to demonstrate any "fasting blood sugar lowering effect" upon chronic administration (6 weeks) of AP. So probably AP prevents glucose absorption from gut. Whole experiment was done on rabbits.

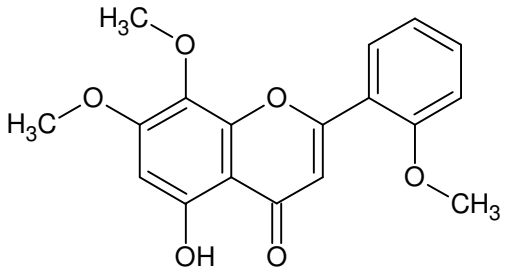
* **S. Rajagopal et al. [2003]** have studied the cellular processes and targets modulated by andrographolide treatment in human cancer and immune cells. Andrographolide treatment inhibited the in vitro proliferation of different tumor cell lines, representing various types of cancers. The compound exerts direct anticancer activity on cancer cells by cell-cycle arrest at G0/G1 phase through induction of cell-cycle inhibitory protein p27 and decreased expression of cyclin-dependent kinase 4 (CDK4). Immunostimulatory activity of andrographolide is evidenced by increased proliferation of lymphocytes and production of interleukin-2. Andrographolide also enhanced the tumor necrosis factor-alpha production and CD marker expression, resulting in increased cytotoxic activity of lymphocytes against cancer cells, which may contribute for its indirect anticancer activity. The in vivo anticancer activity of the compound is further substantiated against B16F0 melanoma syngenic and HT-29 xenograft models. These results suggest that andrographolide is an interesting pharmacophore with anticancer and immunomodulatory activities and hence has the potential for being developed as a cancer therapeutic agent.

STRUCTURES

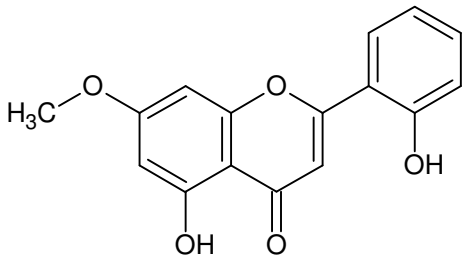
Dihydroechioidinin



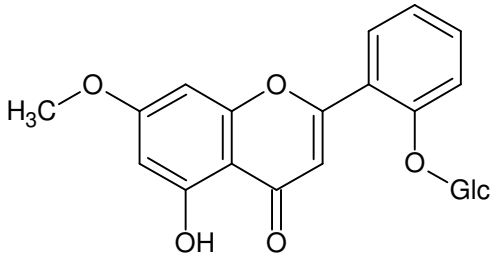
Skullcapflavone I 2'-methyl ether



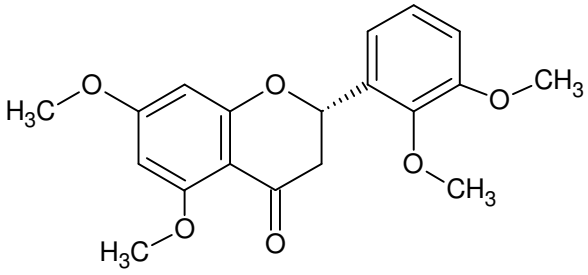
Echioidinin



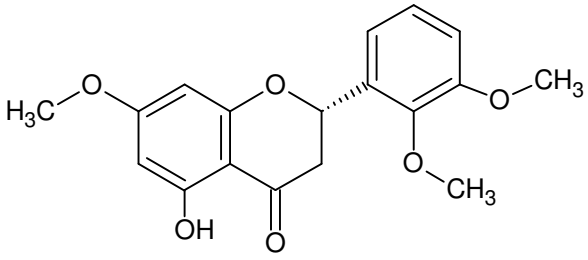
Echioidin



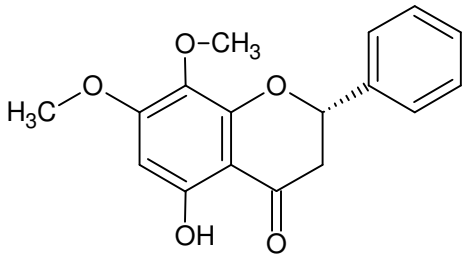
5', 7,2',3' – Tetramethoxy flavanone



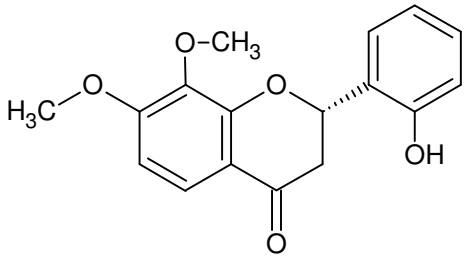
5-hydroxy-7,2',3'-trimethoxyflavone



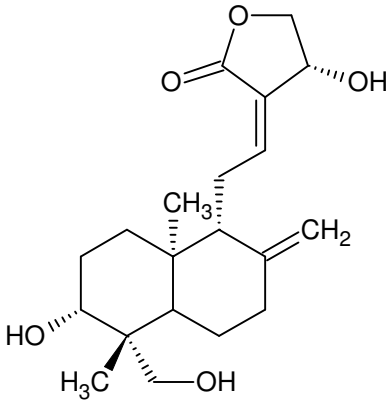
O-methyldihydrowogonin



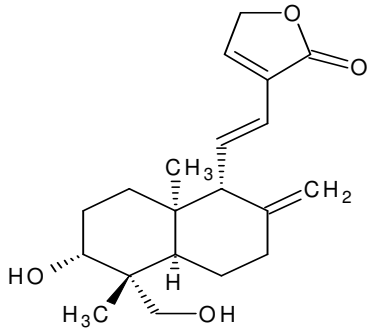
Dihydroskullcapflavone



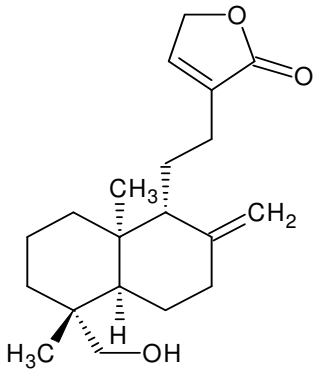
Andrographolide



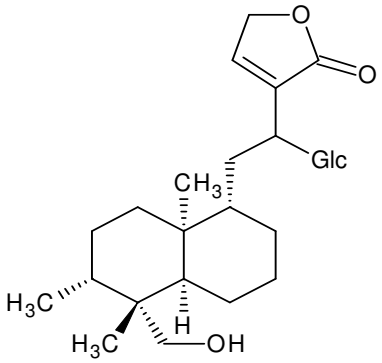
14-Deoxy-11,12-Didehydroandrographolide



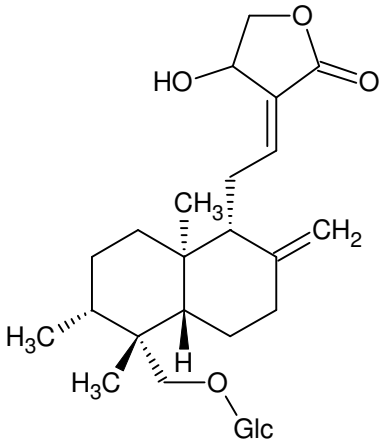
14-Deoxyandrographolide



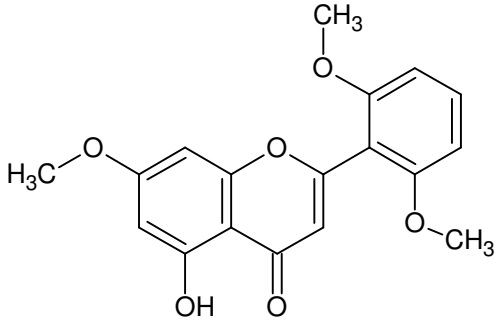
Neo andrographolide



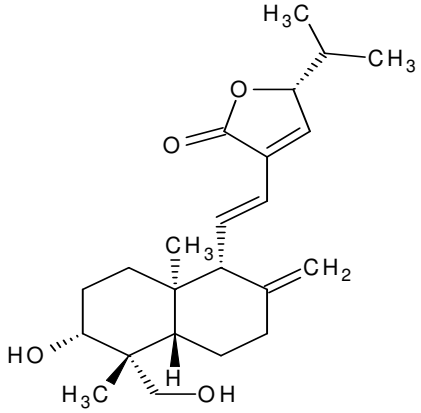
Andrographiside



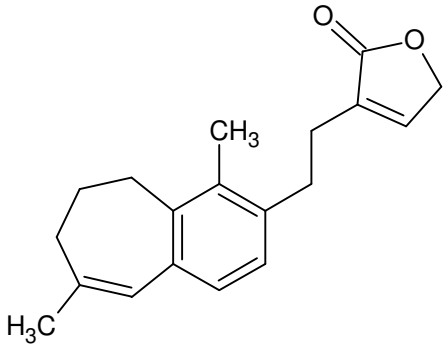
5 - Hydroxy - 7, 2', 6'- trimethoxyflavone



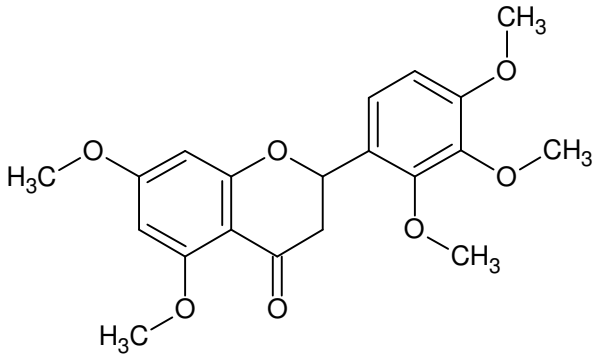
14-Deoxy-15-isopropylidene-11,12- didehydroandrographolide



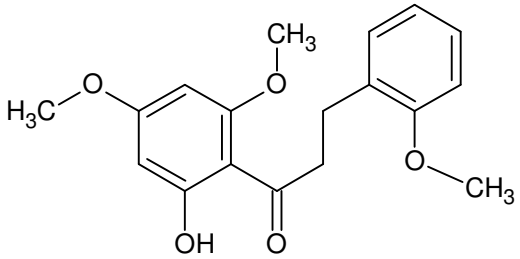
Andrographolactone



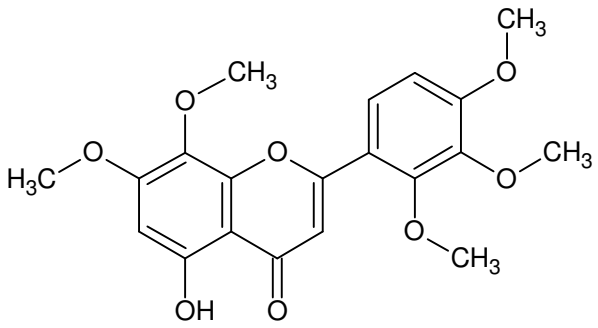
5, 7, 2', 3', 4' – Pentamethoxyflavone



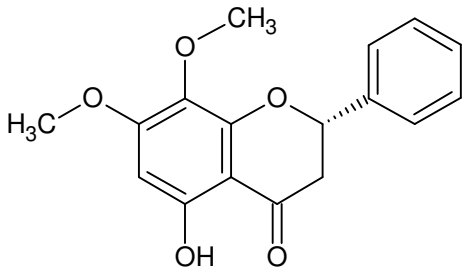
5 – Hydr5oxy – 2, 4', 6' – trimethoxychalcone



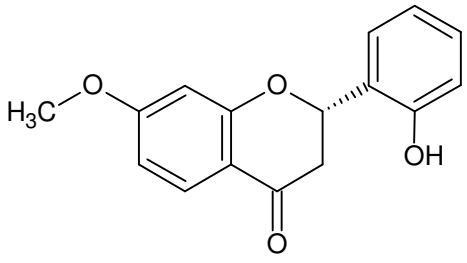
5- Hydroxy - ,7, 8, 2', 3', 4' pentamethoxyflavone



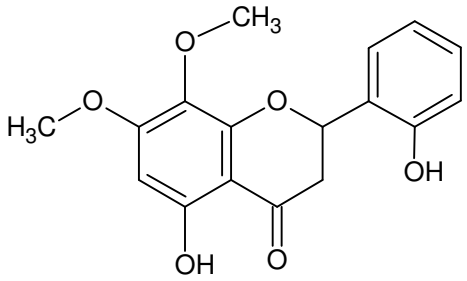
5- Hydroxy – 7, 8 – dimethoxy flavonone



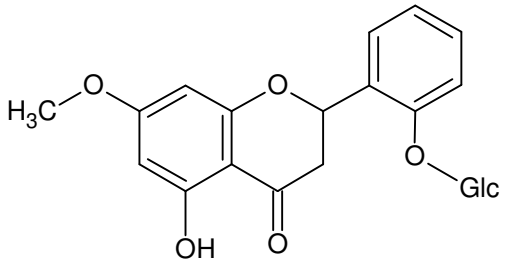
5, 2' – dihydroxy – 7 - methoxy flavanone



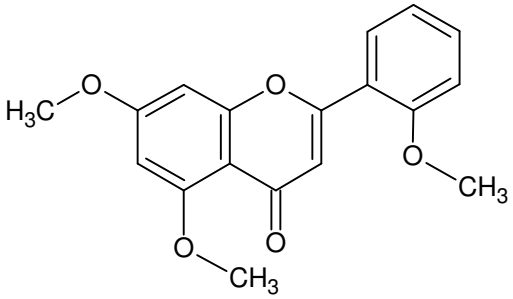
5, 2', dihydroxy – 7, 8 – dimethoxy flavone



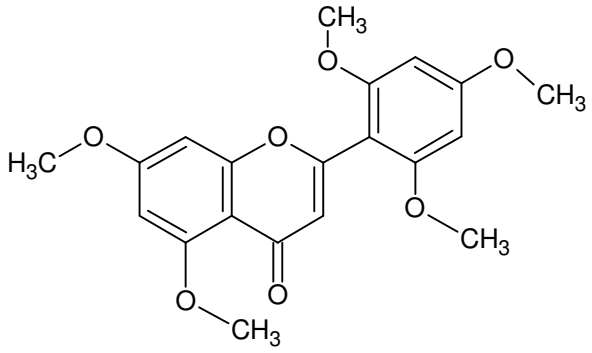
5, 2' – Dihydroxy – 7 – methoxy flavone 2'-O-β-D-glucopyranoside



5, 7, 2' – Trimethoxy flavone



5, 7, 2', 4', 6' – Pentamethoxy flavone



Morphology of Andrographis echioides

PLANT DESCRIPTION [42-46]

BOTONICAL NAME : Andrographis echioides (L) Nees

SYNONYMS : Justicia echioides,

Indoneesiella echioides

VERNACULAR NAMES

A vernacular name of a species can be defined as name that is used generally within a community. It is differentiated with the scientific name for the same species. The synonyms for vernacular names are common name, colloquial name, and popular name. The various vernacular names of the plant ***Andrographis echioides*** are as follows.

- ♣ English : False Water willow
- ♣ Tamil : Gopuram tangi
- ♣ Hindi : Charayetah
- ♣ Malayalam : Pitumba, Mala kulukki
- ♣ Telugu : Chalavala puri kada
- ♣ Marathi : Ranchimani
- ♣ Oriya : Lavalata
- ♣ Gujarati : Kalukariyatun

Andrographis echioides (L) Nees is an annual herb found in many places in India Andrographis It is called as false water willow in English And Gopuram tangi in Tamil.

SCIENTIFIC CLASSIFICATION

Scientific classification of plant can be defined as "The arrangement of entities of that plant" in a hierarchical series of nested classes, in which similar or related classes at one hierarchical level are combined comprehensively into more inclusive classes at the next higher level. The scientific classification of ***Andrographis echioides*** is as follows.

Domain	: Eukaryota
Kingdom	: Plantae
Subkingdom	: Viridaeplantae
Phylum	: Tracheophyta (Vascular Plants)
Subphylum	: Euphyllophytina
Infraphylum	: Radiatopses
Class	: Magnoliopsida (Dicotyledons)
Subclass	: Lamiidae
Superorder	: Lamianae
Order	: Scrophulariales
Family	: Acanthaceae - Acanthus Family
Subfamily	: Acanthoideae
Genus	: Andrographis
Species	: echioides
Botanical name	: Andrographis echioides Nees

Andrographis echioides Nees is an important medicinal plant which belongs to the family Acanthaceae. Some of the species under this family are having similar morphological characters, growth habits, phytochemical constituents, and their

pharmacological activity etc. The different parts of plants in this family consist of flavones and diterpenes as major active constituents.

Andrographis echioides have been reported for their analgesic, anti-inflammatory and antipyretic activity, hepato-protective activity, anti-oxidant and anti-microbial activities. Flavones and flavanoids are the responsible active constituents for mentioned activities.

OTHER SPECIES IN ANDROGRAPHIS:

- ✓ Andrographis affinis
- ✓ Andrographis alata
- ✓ Andrographis atropurpurea
- ✓ Andrographis beddomei
- ✓ Andrographis ceylanica
- ✓ Andrographis elongata
- ✓ Andrographis erpyllifolia
- ✓ Andrographis explicata
- ✓ Andrographis glandulosa
- ✓ Andrographis glomeruliflora
- ✓ Andrographis gracilis
- ✓ Andrographis humifusa
- ✓ Andrographis laxiflora
- ✓ Andrographis lineata
- ✓ Andrographis lobelioides

- ✓ Andrographis macrobotrys
- ✓ Andrographis monglunensis
- ✓ Andrographis nallamalayana
- ✓ Andrographis neesiana
- ✓ Andrographis ovata
- ✓ Andrographis paniculata (creat)
- ✓ Andrographis producta
- ✓ Andrographis rosulata
- ✓ Andrographis rothii
- ✓ Andrographis serpyllifolia
- ✓ Andrographis sinensis
- ✓ Andrographis stellulata
- ✓ Andrographis stenophylla
- ✓ Andrographis subspathulata
- ✓ Andrographis tenera
- ✓ Andrographis tenuiflora
- ✓ Andrographis viscosula
- ✓ Andrographis wightiana

PHYTOCHEMICAL STUDY [47-55]

SOLVENT EXTRACTION:

Collection and Identification of plant:

The details regarding the description of the plant Andrographis echioides have been studied completely. After getting the plant profile, the plant *Andrographis echioides* (Gopuram tangi in Tamil) were collected from the Madurai region during the month of June which was then identified by Dr. Stephen, Lecturer, American college, Madurai. He has given authentification certificate for the plant *Andrographis echioides* after the complete view of fresh plant.

Homogenization:

The fresh plants which have been collected were washed with water to remove soil and other extraneous matter. The leaves of the plants were collected and then cleaned. These cleaned leaves were allowed to dry under shade for 25 days. These dried leaves were then homogenized to coarse powder and stored in an air tight, light resistant container under dark condition.

Apparatus used for extraction and isolation:

Round bottom (RB) flasks, mantle, Bulb condenser, rubber tubes, Adopter, Column, TLC plates, Test tubes, Boiling tubes, Conical flasks, Measuring cylinders, Beakers, Funnel, Watch glass, Thermometer, Capillary tubes, Holder.

Chemicals

- Silica gel, Silica gel G,
- Various chemical reagents,

Solvents:

- ♣ Petroleum ether AR,
- ♣ Hexane AR,
- ♣ Benzene AR,
- ♣ Chloroform AR,
- ♣ Ethyl acetate AR,
- ♣ Methanol AR,
- ♣ Absolute alcohol AR.

Method of Extractions:

About 500g of dried coarse powder of Andrographis echioides leaves were soaked with petroleum ether (3000ml) in RB flask for 3 days. After soaking, they were extracted with petroleum ether by continuous hot percolation method at the temperature of 40-60˚C for 72 hours. The petroleum ether extract was filtered and then concentrated under reduced pressure. A greenish black residue was obtained having the yield of 20g.

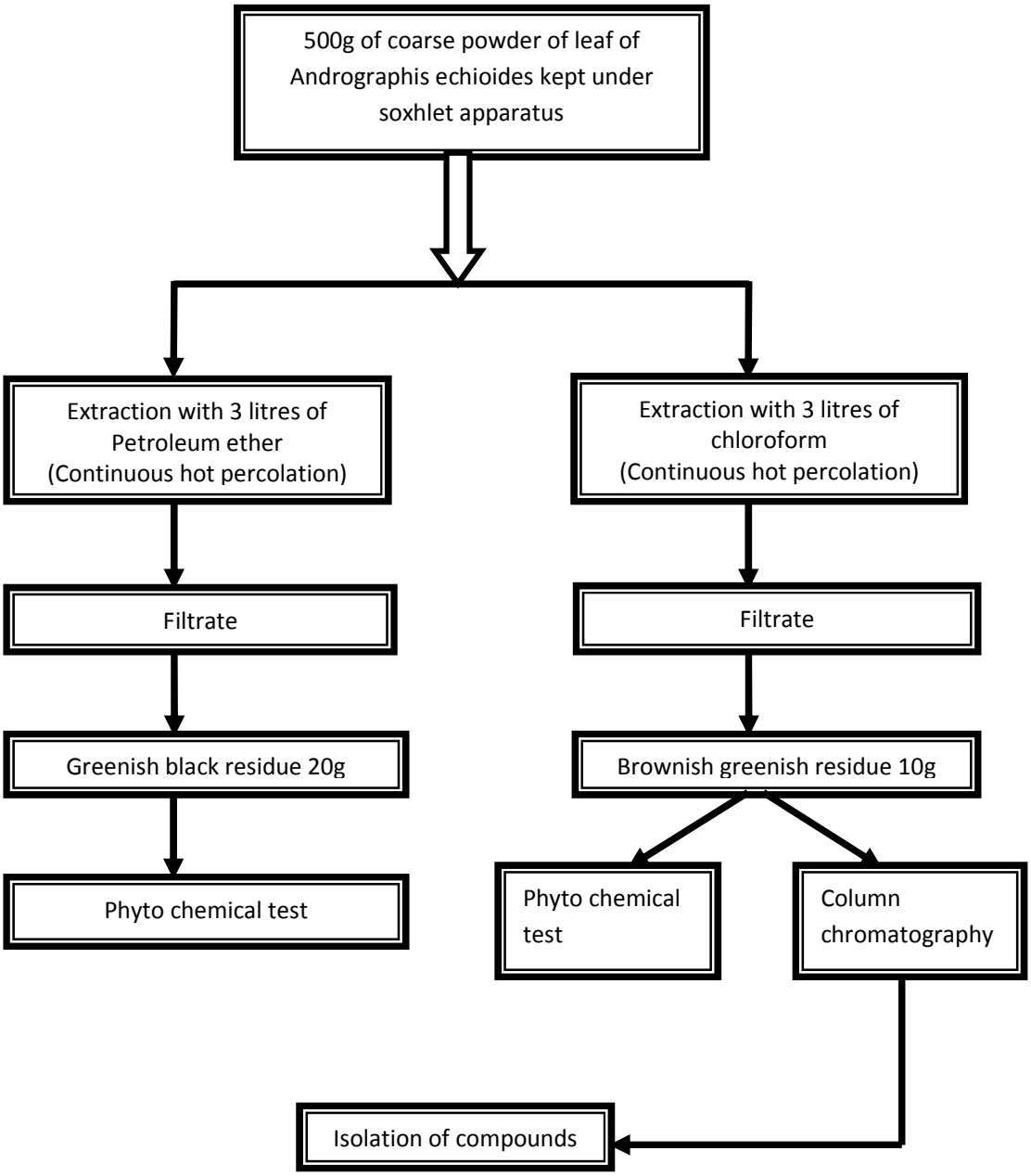
The marc obtained after the extraction of petroleum ether were dried completely and again extracted with the next solvent chloroform (3000ml) by continuous hot percolation method for 72 hours. The chloroform extract also filtered then concentrated under reduced pressure. The color of this extract was brownish green. The yield of this residue was 10g.

Extracts obtained:

- ♥ Petroleum ether extract
- ♥ Chloroform extract

FLOWCHART FOR VARIOUS EXTRACTION AND ISOLATION OF

COMPOUNDS FROM Andrographis echioides L-Nees



PRELIMINARY QUALITATIVE CHEMICAL EVALUATION

The petroleum ether and chloroform extracts of Andrographis echioides were subjected to qualitative tests for identification of various plant constituents.

1. DETECTION OF CARBOHYDRATES:

Small amount of these extracts dissolved in 5ml of chloroform and it was filtered. The filtrate was subjected to Molisch's test to detect the presence of carbohydrate.

- **Molisch's test:**

  Filtrate was treated with 2-3 drops of 1% alcoholic α- naphthol and 2ml of concentrated sulphuric acid was added along the sides of the of the test tube. Violet color ring was formed at the junction of the two liquids from the chloroform extract. This showed the presence of carbohydrates.

2. DETECTION OF GLYCOSIDES:

Small quantity of these two extracts was hydrolyzed with hydrochloric acid for two hours in a water bath. This hydrosylate was subjected to Legal's and Borntrager's test to detect the presence of glycoside.

- **Legal's test:**

  To the hydrosylate of extracts 1ml of pyridine and few drops of sodium nitroprusside solution were added. Then it was made alkaline with sodium hydroxide. Pink to yellow color was obtained in both extracts indicating the presence of glycosides.

- **Borntrager's test:**

  The hydrosylate of extracts was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Pink color was produced in both extracts indicating the presence of glycosides.

3. DETECTION OF PHYTOSTEROLS:

Small quantities of petroleum ether and chloroform extracts were dissolved in 5ml of chloroform separately. These chloroform solution were subjected to Salkowski and Liebermann-Burchard test for the detection of phytosterols.

- **Salkowski test:**

  To the 1ml of prepared chloroform solutions few drops of concentrated sulphuric acid was added. A red color in the lower layer was produced in both extracts which showed the presence of phytosterols in these extracts.

- **Liebermann-Burchard test:**

  The chloroform solutions were treated with few drops of concentrated sulphuric acid followed by 1ml of acetic anhydride solution. A green color was produced in both extracts showed the presence of phytosterols.

4. DETECTION OF SAPONINS:

- The extracts were diluted to the 20ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. The foam was not produced shows the absence of saponins in both extracts.

5. DETECTION OF TANNINS;

- **Gelatin test:**

  Both two extracts were dissolved separately in water and filtered. To the filtrate 1ml of 1% solution of gelatin was added. White precipitate was not produced showed the absence of tannins.

- **Ferric chloride test:**

  Small quantities of these two extracts were dissolved in water separately and to this few drops of ferric chloride solution was added. Black precipitate was not produced indicating the absence of tannins.

6. DETECTION OF PROTEINS AND AMINO ACIDS:

Small quantities of both extracts were dissolved in few ml of water and they were subjected to Millon's, Biuret and Ninhydrin tests.

- **Millon's test:**

  The above prepared solutions of extracts were treated with Millon's reagent and heated. A red color was produced in the chloroform extract indicates the presence of amino acids.

- **Biuret test:**

  To the above prepared solutions of extracts equal volume of 5% sodium hydroxide and 1% Copper sulphate were added. A violet color was produced in chloroform extract indicates the presence of proteins and amino acids.

- **Ninhydrin test:**

  The above prepared solutions of extracts were treated with Ninhydrin reagent. A blue color was produced in the chloroform extract showed the presence of Proteins and Amino acids.

7. DETECTION OF FLAVANOIDS:

- **Shinoda's test:**

  A Small quantity of extracts was dissolved in alcohol and to this magnesium metal followed by concentrated hydrochloric acid was added in drop wise and heated. A magenta color was produced in the chloroform extract indicating the presence of flavanoids.

- **With Ferric chloride:**

  A Small quantity of extracts was dissolved in chloroform. To this, small amount of ferric chloride and potassium ferricyanide were added. A deep blue color was produced in the chloroform extract showed the presence of flavanoids.

8. DETECTION OF FLAVONES:

- **Zinc, Hydrochloric acid reduction test:**

  To the small quantity of these extracts a pinch amount of Zinc dust and few drops of concentrated hydrochloric acid were added. A magenta color was produced in the chloroform extract indicates the presence of flavones.

- **Lead acetate solution test:**

  To a small quantity of both extracts in a few drops of 10% of lead acetate solution was added. Yellow precipitate was produced in chloroform extract showed the presence of flavones.

- **With Sodium hydroxide:**

  A small quantity of extracts was treated with Sodium hydroxide solution. The chloroform extract gave yellow color which showed the presence of flavones.

- **With Concentrated Sulphuric acid:**

  A small quantity of extracts was treated with concentrated sulphuric acid solution. The chloroform extract gave orange color which showed the presence of flavones.

9. DETECTION OF ALKALOIDS:

Small quantity of two extracts was separately treated with few drops dilute hydrochloric acid and filtered. The filtrate was treated with various alkaloidal reagents.

- **Mayer's test:**

  These two extracts were mixed with Mayer's reagent (Potassium mercuric iodide). A Pale yellow precipitate was obtained in chloroform extract which showed the presence of alkaloids.

♣ **Dragondorff's test:**

The extracts were treated with Dragondorff's reagent (Potassium Bismuth iodide). An orange-red color precipitate was obtained in chloroform extract indicated the presence of alkaloids.

♣ **Wagner's test:**

The extracts were treated with Wagner's reagent (Iodine in Potassium iodide). A reddish-brown precipitate was obtained in chloroform extract which showed the presence of alkaloids.

♣ **Hager's test:**

The extracts were treated with Hager's reagent (Saturated aqueous solution of picric acid). A yellow crystalline precipitate was obtained in chloroform extract which showed the presence of alkaloids.

10. DETECTION OF COUMARINS:

♣ **With UV light:**

A small quantity of these extracts was dissolved in alcohol and exposed to UV light. A blue fluorescence was not produced indicated the absence of coumarins.

♣ **With Alcoholic Ferric chloride:**

Both extracts were dissolved separately in alcohol. To this few drops of alcoholic ferric chloride was added. A bluish green color was not obtained indicated the absence of coumarins.

11. DETECTION OF TERPENOIDS:

- **With Antimony trichloride:**

  A small quantity of extracts was mixed with small amount of chloroform then shaked well. To this Antimony trichloride solution was added. A blue color was produced in the chloroform extract indicates the presence of Terpenoidal compounds.

TABLE NO. 1

Data for the preliminary phytochemical screening of the leaf extracts of Andrographis echioides L-Nees

S NO	PHYTO CONSTITUENTS	PETROLEUM ETHER EXTRACT	CHLOROFORM EXTRACT
1	Carbohydrates	-	+
2	Glycosides	+	+
3	Alkaloids	-	+
4	Flavanoids	-	+
5	Flavones	-	+
6	Steroids	+	+
7	Proteins and amino acids	-	+
8	Tannins	-	-
9	Saponins	-	-
10	Coumarins	-	-
11	Terpenoids	-	+

+ indicates presence (positive results)

- indicates absence (negative results)

ISOLATION, PURIFICATION AND IDENTIFICATION OF THE PHYTOCONSTITUENT

Column Chromatography:

After the preliminary chemical tests in the crude extracts, the chloroform extract was selected to perform column chromatography. 6g of chloroform extract was subjected to column chromatography using about 250g of silica gel as a stationary phase.

The silica gel column was packed by using the suspension of silica gel in petroleum ether. Then the chloroform extract was packed by using glass wool. This packed chloroform extract was chromatographed by using different solvent like Petroleum ether, Hexane, Benzene, Chloroform, Ethyl acetate, Methanol and their mixtures in the order of increasing polarity.

The elutes from the column were collected as 100ml, 75ml, 50ml according to the volume of solvent poured. Then these fractions were concentrated and tested for the presence of various constituent. These elutes were also subjected to TLC to find number and type of constituents.

Description of column:

Adsorbent	: Silica gel G (100-200 mesh)
Solvent used for packing	: Petroleum ether
Diameter of the column	: 3cm
Length of the column packing	: 45cm
Amount of chloroform extract	: 10g
Rate of elution	: 25 drops/min

Elutes or column fractions were collected in the conical flasks then they were numbered and kept safely. These elutes were subjected to TLC for identification of each fraction.

THIN LAYER CHROMATOGRAPHY: TLC

Preparation of TLC plates

To prepare TLC plates, Silica gel G was mixed with distilled water in the ratio of (Silica gel G: Water) 1g: 2.5ml. This was then mixed well to form slurry. This slurry was poured on a TLC applicator and then it was adjusted to 0.25mm thickness.

Activation of TLC plate:

The prepared TLC plates were air dried, then kept in the hot air oven at 100˚C for 30 minutes to activate the silica gel G. These plates were then stored in dry atmosphere and used whenever required.

Rf value determination:

The different fractions eluted from the column were spotted on the TLC plates by using capillary tubes. These plates were allowed to run on different solvent system. The spotted compounds were developed according to their affinity towards different solvent system.

The solvent system (Mobile phase in chamber) were allowed to travel $3/4^{th}$ of the TLC plate. Then plates were dried and the spots were identified by using iodine chamber and UV-lamp.

Then finally the Rf values of compounds were calculated by using following formula.

$$\text{Rf value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Purification:

After the TLC, the fractions which are having similar Rf value under same solvent system were grouped together and concentrated. These concentrated fractions were then purified by means of recrystallization by using appropriate solvent.

TABLE NO. 2

Data showing the column chromatography and their elutes of chloroform extract of Andrographis echioides

SOLVENTS	RATIO (ml)	ELUTE NUMBER	COLOUR OF THE ELUTE
Hexane	150	1	Light yellow
Hexane	75	2	Light yellow
Hexane	75	3	Reddish yellow
Hexane	75	4	Yellow
Hexane	75	5	Yellow
Hexane	75	6	Light yellow
Hexane	75	7	Light yellow
Hexane	75	8	Light yellow
Hexane : Benzene	70 : 30	9	Light yellow
Hexane : Benzene	60 : 40	10	Light yellow
Hexane : Benzene	50 : 50	11	Golden yellow
Benzene	100	12	Golden yellow
Benzene	100	13	Light yellow
Benzene : Chloroform	80 : 20	14	Light yellow
Benzene : Chloroform	70 : 30	15	Light yellow
Benzene : Chloroform	60 : 40	16	Light yellow
Benzene : Chloroform	50 : 50	17	Light yellow
Chloroform	50	18	Light yellow
Chloroform	50	19	Light yellow

TABLE NO. 2 (continuations)

Data showing the column chromatography and their elutes of chloroform extract of Andrographis echioides

SOLVENTS	RATIO (ml)	ELUTE NUMBER	COLOUR OF THE ELUTE
Chloroform : Ethyl acetate	80 : 20	20	Greenish brown
Chloroform : Ethyl acetate	70 : 30	21	Brown
Chloroform : Ethyl acetate	60 : 40	22	Dark brown
Chloroform : Ethyl acetate	50 : 50	23	Dark brown
Ethyl acetate	50	24	Dark brown
Ethyl acetate	50	25	Dark brown
Ethyl acetate : Methanol	80 : 20	26	Dark brown
Ethyl acetate : Methanol	70 : 30	27	Light brown
Ethyl acetate : Methanol	60 : 40	28	Light brown
Ethyl acetate : Methanol	50 : 50	29	Light brown
Methanol	50	30	Light brown
Methanol	50	31	Light brown
Methanol	50	32	Light brown
Methanol	50	33	Light brown

TABLE NO. 3

Data showing the column chromatography analysis

S. No	Fraction No	Solvent system for TLC (ml)	Rf value	Solvent used for crystallization	Colour of the compound	Name of the compound
1	30,31,32	Ethyl acetate : methanol 8 : 2	0.80	Chloroform	Greenish Violet	Compound-I (AEA)
2	26,27,28, 29	Ethyl acetate : methanol 8.5 : 1.5	0.68	Chloroform	Dark Green	Compound-II (AEB)
3	22,23,24, 25	Hexane : Ethyl acetate 6 : 4	0.61	Chloroform	Bluish Green	Compound-III (AEC)
4	17,18,19	Hexane : Ethyl acetate 7:3	0.81	Methanol	Greenish Violet	Compound-IV (AEE)
5	12,13,14	Pet. Ether : Ethyl acetate 9:1	0.62	Methanol	Orange Yellow	Compound-V (AEH)

CHARACTERIZATION OF PURIFIED COMPOUNDS BY PHYSICAL, CHEMICAL PROPERTIES AND SPECTRAL DATA

I. COMPOUND 1 -AEA

This compound was obtained from the column elution of chloroform extract of Andrographis echioides.

1. Physical Examination:

Color	: Greenish violet
State	: solid
Yield obtained	: 150mg
Solubility	: Absolute alcohol, Chloroform
Melting point	: 160-162˚C

2. TLC System:

Adsorbent	: Silica gel G
Solvent system	: Ethyl acetate: Methanol (8: 2)
Identification	: UV lamp and Iodine chamber
Rf value	: 0.80

3. Chemical test:

Test for flavones:

- **Zinc, Hydrochloric acid reduction test:**

  To a small quantity of compound 1 (ACE) pinch amount of Zinc dust and few drops of concentrated Hydrochloric acid were added. A magenta color was produced which indicates the presence of flavones.

- **Lead acetate solution test:**

  To a small quantity of compound 1 (ACE) few drops of 10% lead acetate solution was added. A yellow color precipitate was produced which indicates the presence of flavones.

- **With Sodium hydroxide solution:**

  With Sodium hydroxide compound 1 (ACE) gave yellow color, which indicates the presence of flavones.

- **With Concentrated Hydrochloric acid:**

  With concentrated Hydrochloric acid compound 1 (ACE) gave orange color, which indicates the presence of flavones.

4. IR SPECTRAL DATA:

Media: KBr

The IR interpretation of the compound 1 (AEA) is shown in the table.

TABLE NO: 4

IR SPECTRAL DATA OF COMPOUND 1

S No	FREQUENCY cm^{-1}	GROUPS ASSIGNED
1	3042.94	Due to O-H Stretching
2	2920.77	Due to C-H Stretching (Asymmetrical CH_2)
3	2851.11	Due to C-H Stretching (Symmetrical CH_2)
4	1579.30	Due to N-H bending for primary amines
5	1425.74	Due to C-H bending
6	1019.38	Due to C-N Stretching

5. ^{1}H NMR SPECTRAL DATA:

^{1}HNMR spectrum of compound 1 (AEA) was taken using deuteriated chloroform in 300 MHz. Tetra methyl silane (TMS) was used as standard.

^{1}H NMR interpretation of the compound 1 (AEA) is shown in the table;

TABLE NO: 5

^{1}H NMR SPECTRAL DATA OF COMPOUND 1

S.No	SIGNAL VALUES δ ppm	GROUPS ASSIGNED
1	0.856	Due to CH_3 proton
2	1.252	Due to CH_2 proton attached to alkyl group
3	1.593-1.905	Due to CH proton attached to alkyl group
4	2.303	Due to CH_2 proton adjacent to carbonyl group
5	2.777	Due to CH_2 proton adjacent to carbonyl group
6	3.490	Due to CH_2 proton attached to OH group
7	3.665-3.876	Due to CH proton attached to O-R (alkyl group)
8	4.785	Due to alkyl or phenyl esters
9	7.264-7.340	Due to aromatic proton

II. COMPOUND 2 –AEB

This compound was eluted from the column chromatography of chloroform extract of Andrographis echioides

1. Physical Examination:

Color	: Dark green
State	: solid
Yield obtained	: 150mg
Solubility	: Chloroform
Melting point	: 173-175˚C

2. TLC System:

Adsorbent	: Silica gel G
Solvent system	: Ethyl acetate: Methanol (8.5: 1.5)
Identification	: UV lamp and Iodine chamber
Rf value	: 0.68

3. Chemical test:

Detection of flavanoids:

- **Shinoda's test:**

  A Small quantity of the compound II (AEB) was dissolved in alcohol and to this magnesium metal followed by concentrated Hydrochloric acid in drop wise and heated. A magenta color was produced which indicates the presence of flavanoids.

- **With ferric chloride;**

  A small quantity of the Compound II (AEB) was dissolved in chloroform then small amount of ferric chloride and potassium ferricyanide were added. A deep blue color was produced which showed the presence of flavanoids.

4. IR SPECTRAL DATA

Media: KBr

The IR interpretation of the compound 2 (AEB) is shown in the table.

TABLE NO: 6

IR SPECTRAL DATAS OF COMPOUND 2

S No	FREQUENCY cm^{-1}	GROUPS ASSIGNED
1	3402.24	Due to O-H Stretching
2	2920.29	Due to C-H Stretching (Asymmetrical CH_2)
3	2851.03	Due to C-H Stretching (Symmetrical CH_2)
4	1617.32	Due to C=C Stretching
5	1439.29	Due to C-H Bending (sp^3)
6	1234.34	Due to C-O Stretching
7	1160.21	Due to C-O Stretching
8	1074.88	Due to C-O Stretching

5. ^{1}H NMR SPECTRAL DATA:

^{1}HNMR spectrum of compound 2 (AEB) was taken using deuteriated chloroform in 300 MHz. Tetra methyl silane (TMS) was used as standard.

^{1}H NMR interpretation of the compound 2 (AEB) is shown in the table.

TABLE NO: 7

^{1}H NMR SPECTRAL DATA OF COMPOUND 2

S.No.	SIGNAL VALUES δ ppm	GROUPS ASSIGNED
1	0.879-0.969	Due to CH_3 proton
2	1.253	Due to CH_2 proton attached to alkyl group
3	1.598	Due to CH proton attached to C=C group
4	2.063	Due to CH proton attached to C=C group
5	2.208-2.291	Due to CH_2 proton adjacent to C=O group
6	2.802	Due to CH_2 proton adjacent to C=O group
7	3.863	Due to CH proton attached to OH group
8	7.266-7.304	Due to aromatic proton

III. COMPOUND 3 –AEC

This compound was eluted from the column chromatography of chloroform extract of Andrographis echioides

1. Physical Examination:

Color	: Bluish green
State	: Semi solid
Yield obtained	: 100mg
Solubility	: Chloroform and Methanol
Melting point	: 203-205˚C

2. TLC System:

Adsorbent	: Silica gel G
Solvent system	: Ethyl acetate: Methanol (6: 4)
Identification	: UV lamp and Iodine chamber
Rf value	: 0.61

3. Chemical test:

Test for Terpenoids:

- **With Antimony trichloride:**

  A small quantity of Compound III (AEC) was mixed with small amount of chloroform then shaked well. To this Antimony trichloride was added. A blue color was produced in the chloroform extract indicates the presence of Terpenoidal compounds.

4. IR SPECTRAL DATA:

Media: KBr

The IR interpretation of the compound 3 (AEC) is shown in the table.

TABLE NO: 8

IR SPECTRAL DATAS OF COMPOUND 3

S No	FREQUENCY cm^{-1}	GROUPS ASSIGNED
1	3396.76	Due to O-H Stretching
2	2920.32	Due to C-H Stretching (Asymmetrical CH_2)
3	2852.81	Due to C-H Stretching (Symmetrical CH_2)
4	1737.92	Due to C=O Stretching (carbonyl group)
5	1710.92	Due to C=O Stretching (carbonyl group)
6	1654.98	Due to N-H Bending (primary amine)
7	1454.38	Due to C-H Bending (Sp3)
8	1348.29	Due to C-N stretching (Aromatic amines)
9	1230.63	Due to C-O stretching
10	1159.26	Due to C-O stretching
11	972.16	Due to C-H bending-opposite (olefins)
12	906.57-678.97	Due to N-H bending-opposite (amines)

5. ^{1}H NMR SPECTRAL DATA:

^{1}HNMR spectrum of compound 3 (AEC) was taken using deuteriated chloroform in 300 MHz. Tetra methyl silane (TMS) was used as standard.

^{1}H NMR interpretation of the compound 3 (AEC) is shown in the table.

TABLE NO: 9

^{1}H NMR SPECTRAL DATA OF COMPOUND 3

S.No	SIGNAL VALUES δ ppm	GROUPS ASSIGNED
1	0.834-1.002	Due to CH_3 proton
2	1.253	Due to CH_2 proton attached to alkyl group
3	1.315	Due to CH_2 proton attached to –C-C- group
4	1.598- 2.099	Due to CH proton attached to C=C group
5	2.346	Due to CH_2 proton attached to C=O group
6	7.263	Due to aromatic proton

IV. COMPOUND 4-AEE

This compound was eluted from the column chromatography of chloroform extract of leaves of Andrographis echioides

1. Physical Examination:

Color	: Light greenish violet
State	: Semi solid
Yield obtained	: 100mg
Solubility	: Chloroform and Methanol
Melting point	: 176-178˚C

2. TLC System:

Adsorbent	: Silica gel G
Solvent system	: Ethyl acetate: Methanol (7: 3)
Identification	: UV lamp and Iodine chamber
Rf value	: 0.81

3. Chemical test:

Test for flavones:

- **Zinc, Hydrochloric acid reduction test:** To a small quantity of compound IV (AEE), pinch amount of Zinc dust and few drops of concentrated Hydrochloric acid were added. A magenta color was produced which indicates the presence of flavones.
- **Lead acetate solution test:** To a small quantity of compound IV (AEE) few drops of 10% lead acetate solution was added. A yellow color precipitate was produced which indicates the presence of flavones.
- **With Sodium hydroxide solution:** With Sodium hydroxide compound IV (AEE) gave yellow color, which indicates the presence of flavones.
- **With Concentrated Hydrochloric acid:** With concentrated Hydrochloric acid compound IV (AEE) gave orange color, which indicates the presence of flavones.

4. IR SPECTRAL DATA:

Media: KBr

The IR interpretation of the compound 4 (AEE) is shown in the table.

TABLE NO: 10

IR SPECTRAL DATAS OF COMPOUND 4

S No	FREQUENCY cm^{-1}	GROUPS ASSIGNED
1	3421.96	Due to O-H Stretching
2	2919.39	Due to C-H Stretching (asymmetrical CH_2)
3	2850.81	Due to C-H Stretching (symmetrical CH_2)
4	1619.04	Due to C=C Stretching
5	1440.06	Due to C-H bending (sp^3)
6	1157.87	Due to C-O Stretching
7	1020.33	Due to C-O Stretching

5. ^{1}H NMR SPECTRAL DATA:

^{1}HNMR spectrum of compound 4 (AEE) was taken using deuteriated chloroform in 300 MHz Tetra methyl silane (TMS) was used as standard.

^{1}H NMR interpretation of the compound 4 (AEE) is shown in the table.

TABLE NO: 11

^{1}H NMR SPECTRAL DATA OF COMPOUND 4

S.No	SIGNAL VALUES δ ppm	GROUPS ASSIGNED
1	0.836-1.002	Due to CH_3 proton
2	0.254-1.279	Due to CH_2 proton attached to alkyl group
3	1.602-2.058	Due to CH proton attached to C=C group
4	2.346	Due to CH_2 proton adjacent to C=O group
5	3.687-3.820	Due to CH proton attached to OR
6	7.262-7.287	Due to aromatic protons

V. COMPOUND 5 –AEH

This compound was eluted from the column chromatography of chloroform extract of leaves of Andrographis echioides

1. Physical Examination:

Color	: Yellowish orange
State	: Semi solid
Yield obtained	: 150mg
Solubility	: Ethyl acetate, Chloroform and Methanol
Melting point	: 170-173˚C

2. TLC System:

Adsorbent	: Silica gel G
Solvent system	: Petroleum ether: Ethyl acetate (9: 1)
Identification	: UV lamp and Iodine chamber
Rf value	: 0.62

3. Chemical test:

Test for Terpenoids:

♣ **With Antimony trichloride:**

A small quantity of Compound V (AEH) was mixed with small amount of chloroform then shaked well. To this Antimony trichloride was added. A blue color was produced in the chloroform extract indicated the presence of Terpenoidal compounds.

4. IR SPECTRAL DATA:

Media: KBr

The IR interpretation of the compound 5 (AEH) is shown in the table.

TABLE NO: 12

IR SPECTRAL DATAS OF COMPOUND 5

S No	FREQUENCY cm^{-1}	GROUPS ASSIGNED
1	3034.13	Due to C-H Stretching (Olefines)
2	2922.25	Due to C-H Stretching (Asymmetrical CH_2)
3	2852.81	Due to C-H Stretching (Symmetrical CH_2)
4	2727.44	Due to C-H Stretching (Aldehyde)
5	1735.999	Due to C=O Stretching (Aldehyde)
6	1662.69	Due to C=C Stretching (Olefines)
7	1448.59	Due to C-H Bending (sp^3)
8	1375.29	Due to C-H Bending
9	1257.29	Due to C-N Stretching (Aromatic amines)
10	1168.90	Due to C-O Stretching
11	1089.82	Due to C-O Stretching
12	835.21	N-H Bending (Opposite)
13	738.76	Due to C-H Bending (Opposite)

5. ^{1}H NMR SPECTRAL DATA:

^{1}HNMR spectrum of compound 5 (AEH) was taken using deuteriated chloroform in 300 MHz Tetra methyl silane (TMS) was used as standard.

^{1}H NMR interpretation of the compound 5 (AEH) is shown in the table.

TABLE NO: 13

^{1}H NMR SPECTRAL DATA OF COMPOUND 5

S.No	SIGNAL VALUES δ ppm	GROUPS ASSIGNED
1	0.855-0.892	Due to CH_3 proton
2	1.253-1.365	Due to CH_2 proton attached to alkyl group
3	1.508-1.677	Due to CH proton attached to alkyl group
4	1.712-2.149	Due to CH proton attached to C=C group
5	2.293	Due to CH_2 proton adjacent to C=O group
6	5.126-5.213	Due to CH_2 proton attached to ethylenic double bond
7	7.260-7.373	Due to aromatic proton

TABLE NO.14

^{13}C NMR SPECTRAL DATA OF COMPOUND 5 (AEH)

S.No	SIGNAL VALUES (δ) ppm
1	14.046
2	15.995
3	19.733
4	22.656
5	23.379-23.429
6	24.452-25.643
7	29.364-29.685
8	31.930-32.800
10	34.415
11	37.455
12	39.389-39.742
13	76.586-77.425
14	124.309
15	125.053
16	135.191

DIURETIC ACTIVITY OF VARIOUS EXTRACT OF ANDROGRAPHIS ECHIOIDES [56-59]

INTRODUCTION

Diuretics are the drugs capable of increasing the rate of urine flow and sodium excretion and are used to adjust the volume and composition of body fluids in a variety of clinical situations, including hypertension, heart failure, renal failure, nephritic syndrome and cirrhosis.

These diuretic drugs act on the kidney and are able to increase the volume of urine excretion. The urine output will be increased after the administration of diuretic drugs like frusemide. [1]

Factors governing urine outflow

The following phenomenons in renal system are the major factors which are regulate the urine outflow and electrolyte contents of the intra and extra cellular fluids.

- ♣ Glomerular filtration rate
- ♣ Tubular reabsorption
- ♣ Tubular secretion

Thus renal outflow is regulated by these factors. The volume and contents of intra and extra cellular fluids are balanced by hormones namely Aldosterone and vasopressin (ADH).

Classification of diuretics

Diuretics are used under such conditions like imbalance of renal outflow.

These drugs are used to treat some critical states such as renal failure, hypertension, heart failure cirrhosis etc.

These diuretics can be classified as follow

Weak diuretics

- ♣ Osmotic diuretics- sodium and potassium salts
- ♣ Xanthine derivatives- aminophylline
- ♣ Carbonic anhydrase inhibitors- acetazolamide

Moderately efficacious diuretics

- ♣ Osmotic diuretics- mannitol, isosorbide
- ♣ Benzothiadiazines- chlorthalidone

Very efficacious diuretics (high ceiling diuretics)

- ♣ Frusemide,
- ♣ Mefruside

Potassium sparing diuretics

- ♣ Aldosterone antagonists- spiranolactone

These are about the classification of diuretics.

Since the diuretic activity of this plant *Andrographis echioides* has not been scientifically evaluated, the present study was undertaken to investigate the effect of petroleum ether and chloroform extract of *Andrographis echioides* for its diuretic activity with their electrolyte excretion. [2]

Materials and methods

Animals

Adult male Wistar rats, each in the weight range of 180-200g, were obtained from the animal house, K.M.College of pharmacy, Madurai. The animals were randomly allocated to four treatment groups of six animals each and kept in poly propylene cages and housed under standard conditions of temperature, humidity and dark light cycle (12h-12h).

Diuretic activity:

The Wistar rates were divided into four of six animals each.

Group I- served as Normal control and received normal saline orally.

Group II- served as positive control and received frusemide (20mg/kg)

Group III- served as treatment control received 200mg/kg of petroleum ether exgtract of *Andrographis echioides*.

Group IV- served as treatment control received 200mg/kg of chloroform extract of *Andrographis echioides.*

Immediately after administration the animals were placed in metabolic cages spirally designed to separate urine and faeces at room temperature of 25 ± 0-5°C.

The observed parameters were total volume, Na^+, K^+ and Cl^- excreted in the urine. The concentration of sodium and potassium ions were measured by flame photometer and chloride ion concentration was estimated by titration with silver nitrate solution (N/50) using three drops of potassium chromate as an indicator. [3], [4]. Data are presented as Mean ± SEM.

Statistics:

Statistically, the values were analyzed with the analysis of variance (one way ANOVA) followed by Newman kevly multiple range tests to determine the significance of difference within the experimental groups.

TABLE NO. 15

ELECTROLYTE EXCRETION AND DIURETIC ACTIVITY OF VARIOUS EXTRACTS OF ANDROGRAPHIS ECHIOIDES

Groups	Treatment	Dose	Volume of urine (ml)	Electrolyte excretion		
				Na^+ m.eq/L	K^+ m.eq/L	Cl^- m.eq/L
Group-I	Normal control	10ml/kg	7.6 ±0.60	63.85 ±3.93	12.15 ±0.68	52.98 ±2.95
Group-II	Positive control. frusemide	20mg/kg	14.2 ±1.06	126.26 ±6.24	17.36 ±1.10	110.6 ±4.10
Group-III	Treatment control. Pet. Ether extract	200mg/kg	8.8 ±0.78	85.93 ±4.26	13.76 ±0.88	73.33 ±3.68
Group-IV	Treatment control. chloroform extract	200mg/kg	12.50 ±0.98	103.45 ±5.50	14.96 ±0.96	94.9 ±3.95

Values are expressed as Mean ± SEM

Values were found out by using one way ANOVA followed by Newman Keul's multiple range tests

Values were significantly different from normal control at $P<0.01$

BAR CHARTS FOR DIURETIC ACTIVITY

GROUPS Vs PARAMETERS

CHART NO: 1

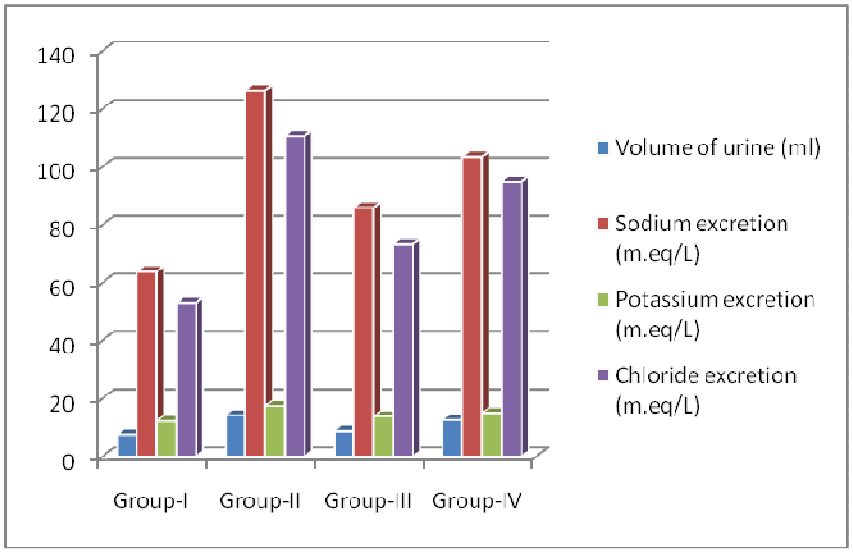
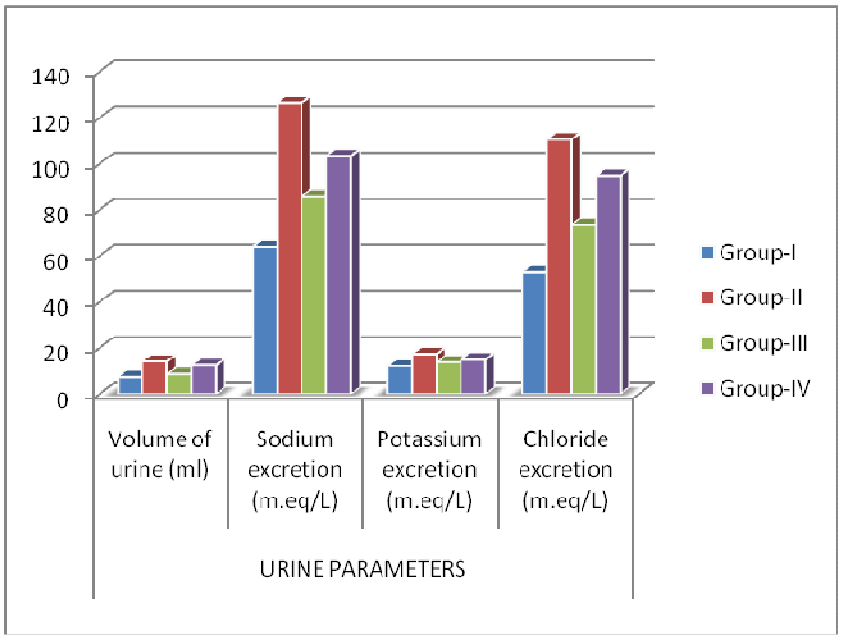


CHART NO: 2



Diuretic potential

The data showed that, the chloroform extract of *Andrographis echioides* produced significant diuretic activity, evidenced by the increased excretion of sodium and potassium ions, comparable to the standard drug, frusemide. Hence I concluded that the chloroform extract of *Andrographis echioides* showed effective diuretic activity by increasing the total urine output and increased excretion of sodium and potassium salts.

RESULT AND DISCUSSION

After preliminary phyto chemical screening of the leaf extracts, it was decided to isolate and characterize the chemical constituents of chloroform extract by column chromatography. These extracts were also selected to study the diuretic activity. In the preliminary screening, these extracts showed the presence of constituents like carbohydrates, alkaloids, glycosides, flavanoids, steroids, proteins and amino acids and terpenes.

In the column chromatography of chloroform extract which was carried out by using silica gel 100 – 200 mesh. Five compounds namely AEA, AEB, AEC, AEE and AEH were obtained as a column elute in the solvents with the increasing order of polarity i.e. Petroleum ether, Hexane, Benzene, Chloroform, Ethyl acetate and Methanol.

Compound AEA showed greenish violet in appearance which is in solid state. The melting point of this compound was 160-162˚C. It was soluble in chloroform and alcohol. On TLC, AEA showed single spot having the solvent system of Ethyl acetate: Methanol (8:2). The Rf value of this compound was 0.80.

The IR data showed frequency cm^{-1} for particular functional group at 3402, 2920, 2851, 1579, 1425, 1019 cm^{-1}.

The ^{1}H NMR spectra showed the signals at 0.856, 1.252, 1.593 - 1.905, 2.303, 2.777, 3.490, 3.665 - 3.876, 4.785, 7.264 - 7.340 δ ppm.

These data showed that this Compound AEA may be a **Flavanoid** type which was confirmed by chemical test [12].

Compound AEB showed dark green color in appearance which is solid state. The melting point was 173-175˚C. Soluble in chloroform, a single spot was obtained for this compound on TLC having the solvent system of Ethyl acetate: Methanol (8.5: 1.5) having the Rf value of 0.68.

The IR spectra showed frequency cm^{-1} at 3402, 2920, 2851, 1617, 1439, 1234, 1160, 1074 cm^{-1} for their respective functional groups.

The ^{1}H NMR spectra showed signals δ ppm at 0.879 – 0.969, 1.253, 1.598, 2.063, 2.208 – 2.291, 2.802, 3.863, 7.266 – 7.304 δ ppm.

The above data showed that this compound AEB may be a **Flavanoid** type of compound which was further confirmed by chemical test [16].

Compound AEC showed bluish green in appearance which is semisolid state. The melting point of this compound was 203 - 205˚C and soluble in chloroform and methanol. Single spot was detected on TLC plate by using Hexane: Ethyl acetate (6:4) as solvent system. The Rf value of this compound was 0.61.

The IR spectrum of AEC showed frequency cm^{-1} at 3396, 2920, 2852, 1737, 1710, 1654, 1454, 1348, 1230, 1159, 972, 906 – 678 cm^{-1} which are responsible for characteristic functional group.

The ^{1}H NMR spectra showed signals at 0.834-1.002, 1.253, 1.315, 1.598 – 2.099, 2.346, 3.81, 5.4, 7.263 δ ppm.

The above said data showed that this compound may be **Diterpene** type of compound which was confirmed by chemical test [14].

Compound AEE showed light greenish violet in appearance which is in semisolid state. The melting point of this compound was 176-178˚ C. It was soluble in chloroform and methanol. It gives a single spot on TLC using Hexane: Ethyl acetate (7: 3) as solvent system. The Rf value of the spot was calculated as 0.81.

The IR spectrum of AEE showed frequency cm^{-1} values at 3421, 2919, 2850, 1619, 1440, 1157, 1020 cm^{-1} which are responsible for the characteristic functional groups.

The ^{1}H NMR spectra showed chemical signals δ ppm at 0.836 – 1.002, 0.254 – 1.279, 1.602 – 2.058, 2.346, 3.687 – 3.820, 7.262 – 7.287 δ ppm.

The above data showed that the compound AEE may be a **Flavone** type of compound which is confirmed by chemical test [17].

Compound AEH showed yellowish orange in appearance which was semisolid in nature. The melting point of this compound was 170-173˚C which is soluble in Ethyl acetate, Methanol and Chloroform. TLC showed single spot by using Petroleum ether: Ethyl acetate (9: 1) as solvent system. The Rf value of this compound is 0.62.

The IR spectrum of compound AEH showed frequency cm^{-1} values at 3034, 2922, 2852, 2727, 1735, 1662, 1448, 1375, 1257, 1168, 1089, 835, 738 cm^{-1} which are responsible for their characteristic functional groups.

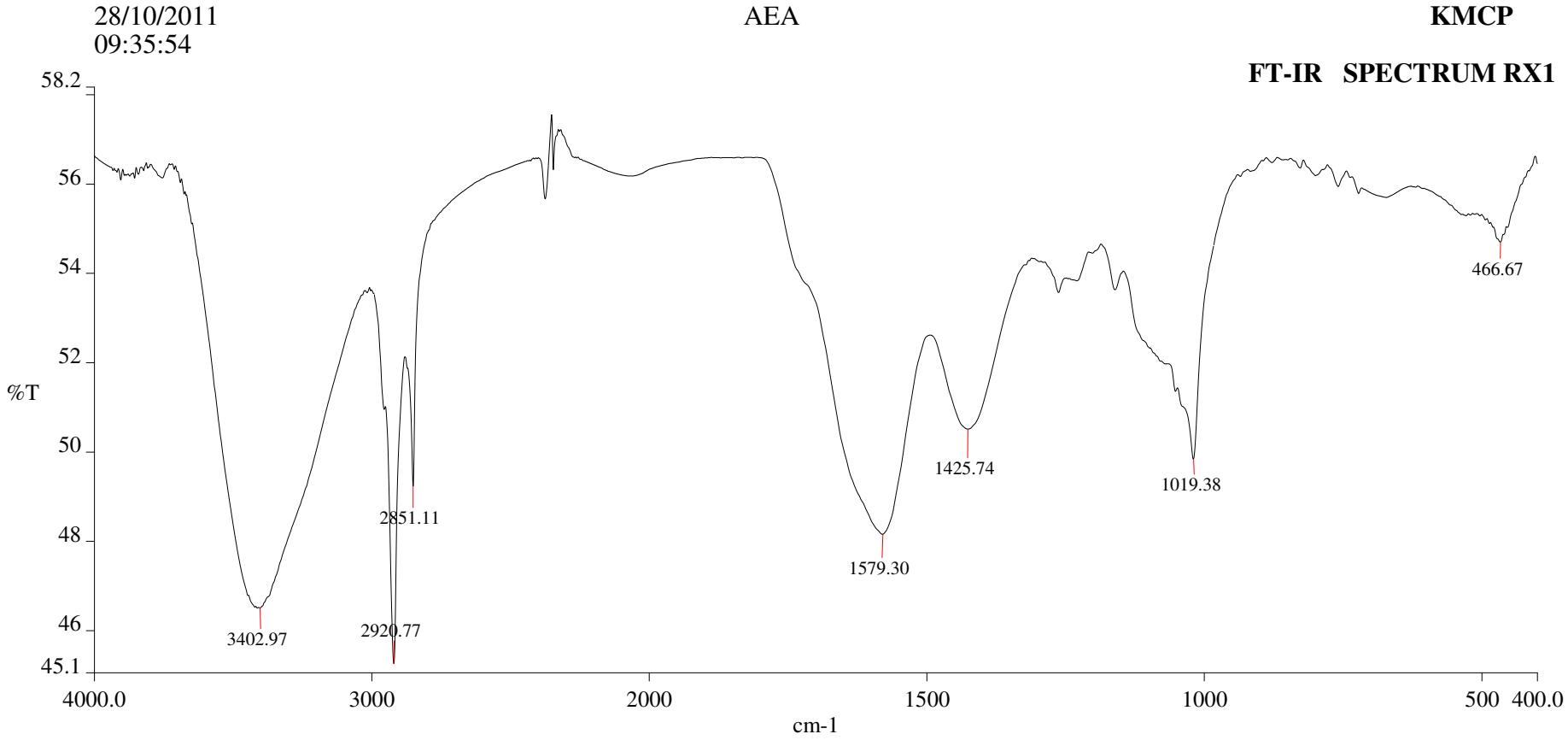
The ^{1}H NMR spectra showed signal δ ppm at 0.855 – 0.892, 1.253 – 1.365, 1.508 – 1.677, 1.712 – 2.149, 2.293, 5.126 – 5.213, 7.260 – 7.373.

The ^{13}C NMR showed values at 14.046, 15.995, 19.733, 22.656, 23.379 - 23.429, 24.452 – 25.643, 26.415 – 27.981, 29.364 – 29.685, 31.930 – 32.800, 34.415, 37.455, 39.389 – 39.742, 76.586 – 77.425, 124.309, 125.053, 135.191.

The above data showed that the compound AEH may be a **Terpenoidal** type of compound which was confirmed by chemical test [13].

Regarding to the diuretic activity, the chloroform extract of leaf powder of the *Andrographis echioides* has shown better activity than Petroleum ether extract. It also has significant activity when compared to the standard drug frusemide.

This significant diuretic activity evidenced by increased excretion of sodium and potassium salts as well as the volume urine output hence it was concluded that chloroform extract of leaf powder of *Andrographis echioides* posses diuretic action.

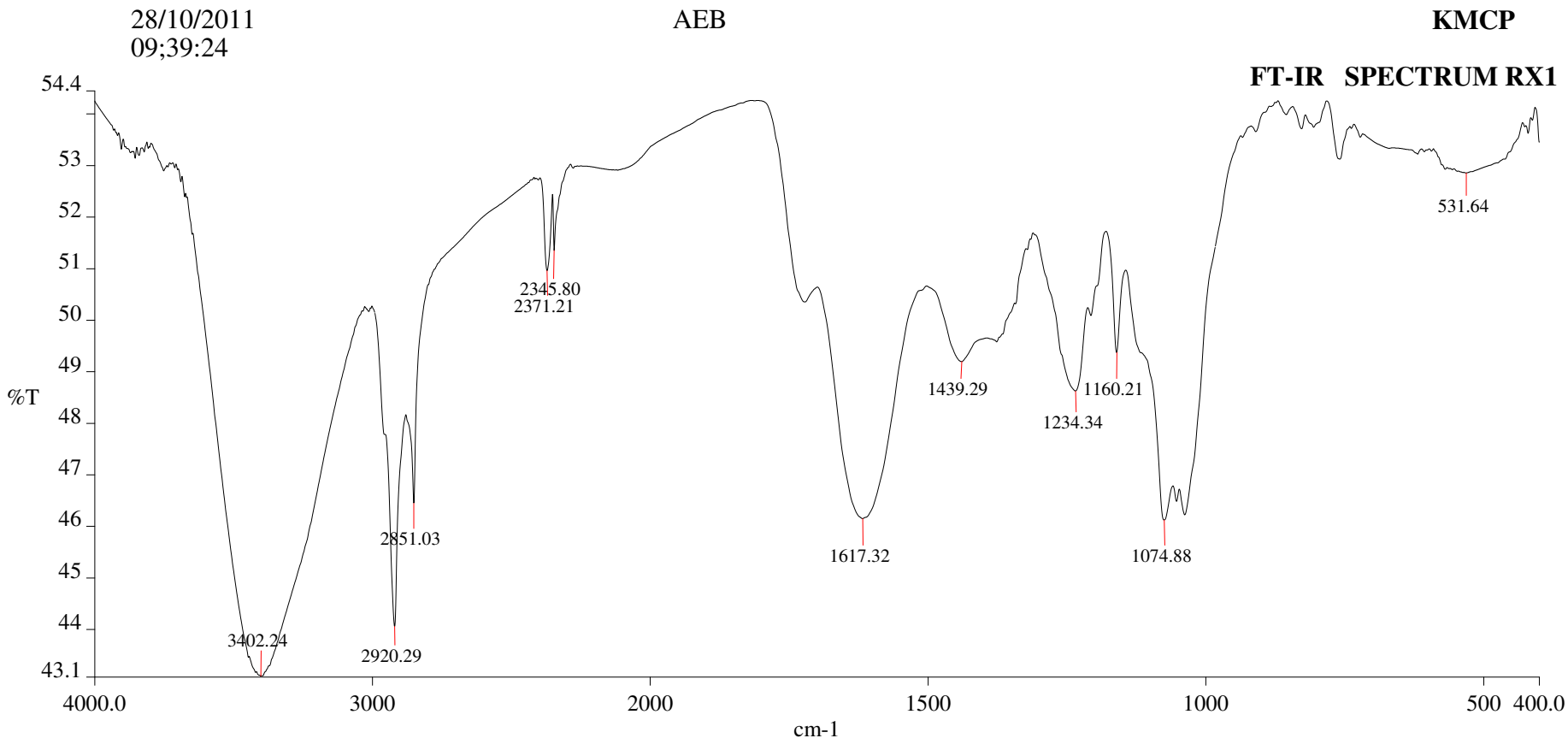


Spectrum Pathname: C:\PEL_DATA\SPECTRA\aea.002

aea.pk

aea.002 3601 4000.00 400.00 45.26 57.55 4.00 %T 5 1.00

REF 4000 56.63 2000 56.33 600
3402.97 46.50 2920.77 45.26 2851.11 49.23 1579.30 48.15 1425.74 50.51
1019.38 49.83 466.67 54.69



Spectrum Pathname: C:\PEL_DATA\SPECTRA\aeb.004

aeb.pk

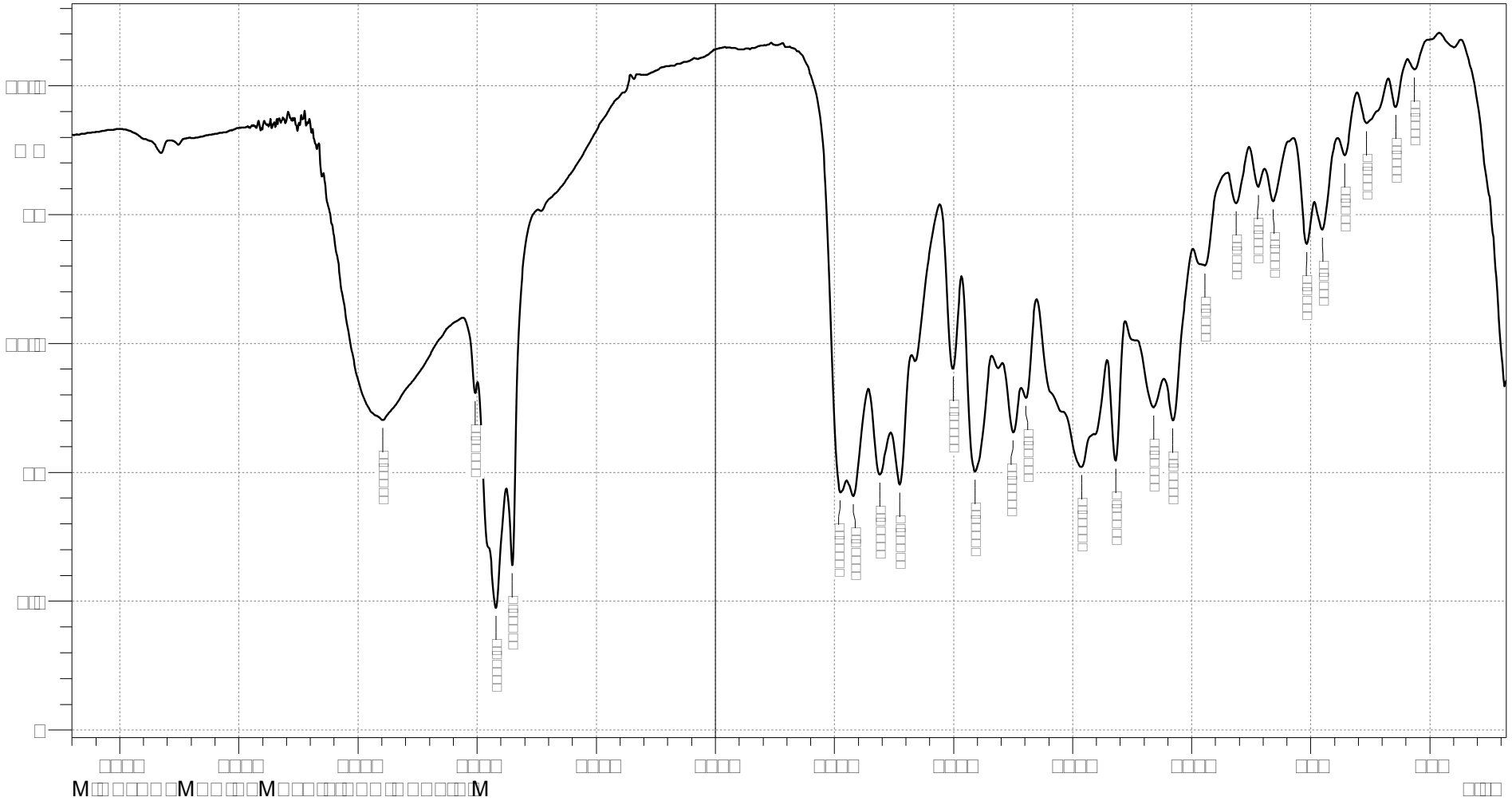
aeb.004 3601 4000.00 400.00 43.09 54.26 4.00 %T 5 1.00

REF 4000 54.24 2000 53.36 600

3402.24 43.09 2920.29 44.05 2851.03 46.45 2371.21 50.95 2345.80 51.35
1617.32 46.15 1439.29 49.19 1234.34 48.62 1160.21 49.37 1074.88 46.12
531.64 52.85

USIC-MKU

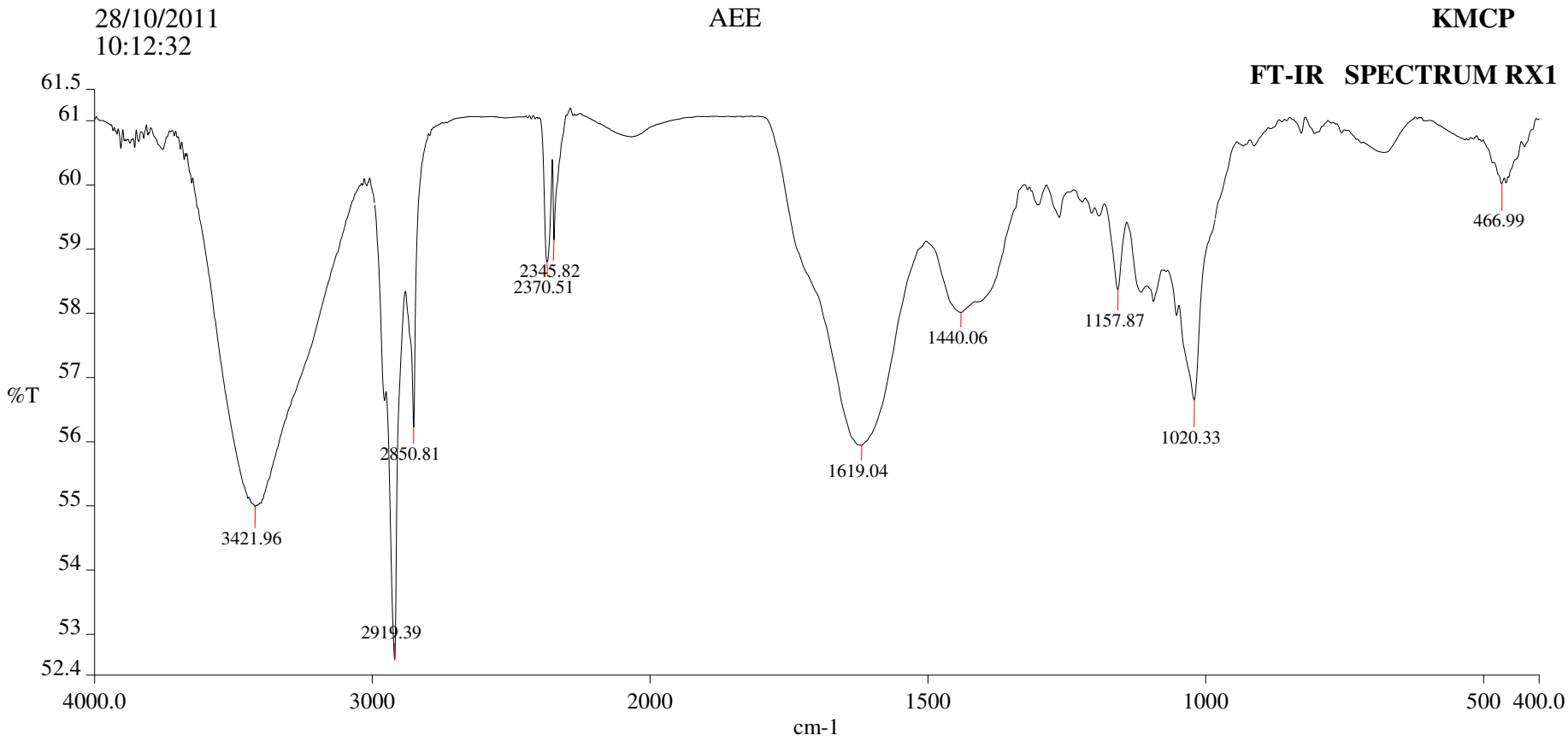
SHIMADZU



Comment;

Resolution;
Apodization;

Date/Time; 11/23/2011 12:25:51 PM
User; USIC



Spectrum Pathname: C:\PEL_DATA\SPECTRA\aee.002

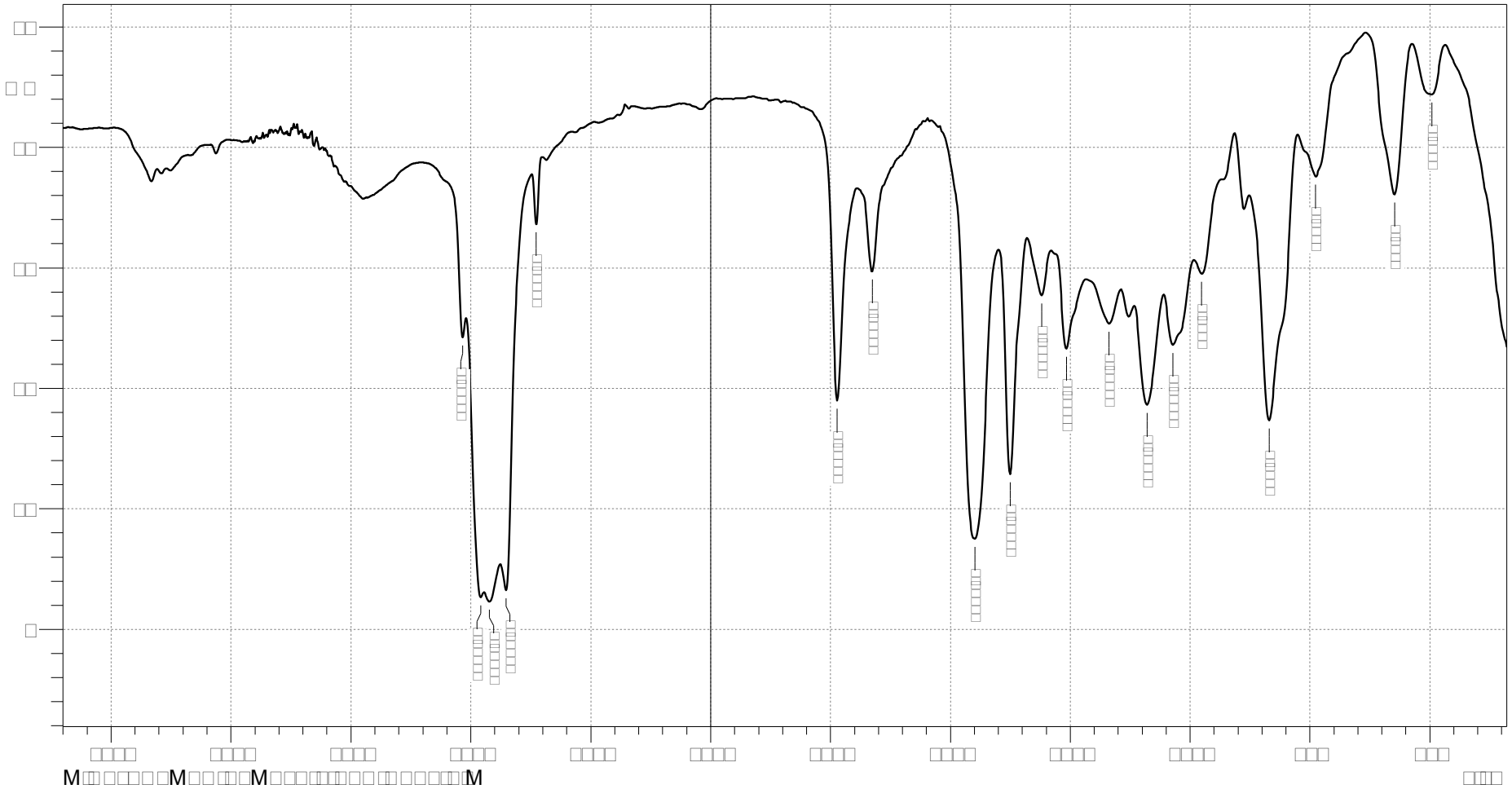
aee.pk

aee.002 3601 4000.00 400.00 52.61 61.19 4.00 %T 5 1.00

REF 4000 61.02 2000 60.89 600

3421.96 54.98 2919.39 52.60 2850.81 56.21 2370.51 58.80 2345.82 59.14
1619.04 55.94 1440.06 58.01 1157.87 58.37 1020.33 56.65 466.99 60.01

USIC-MKU

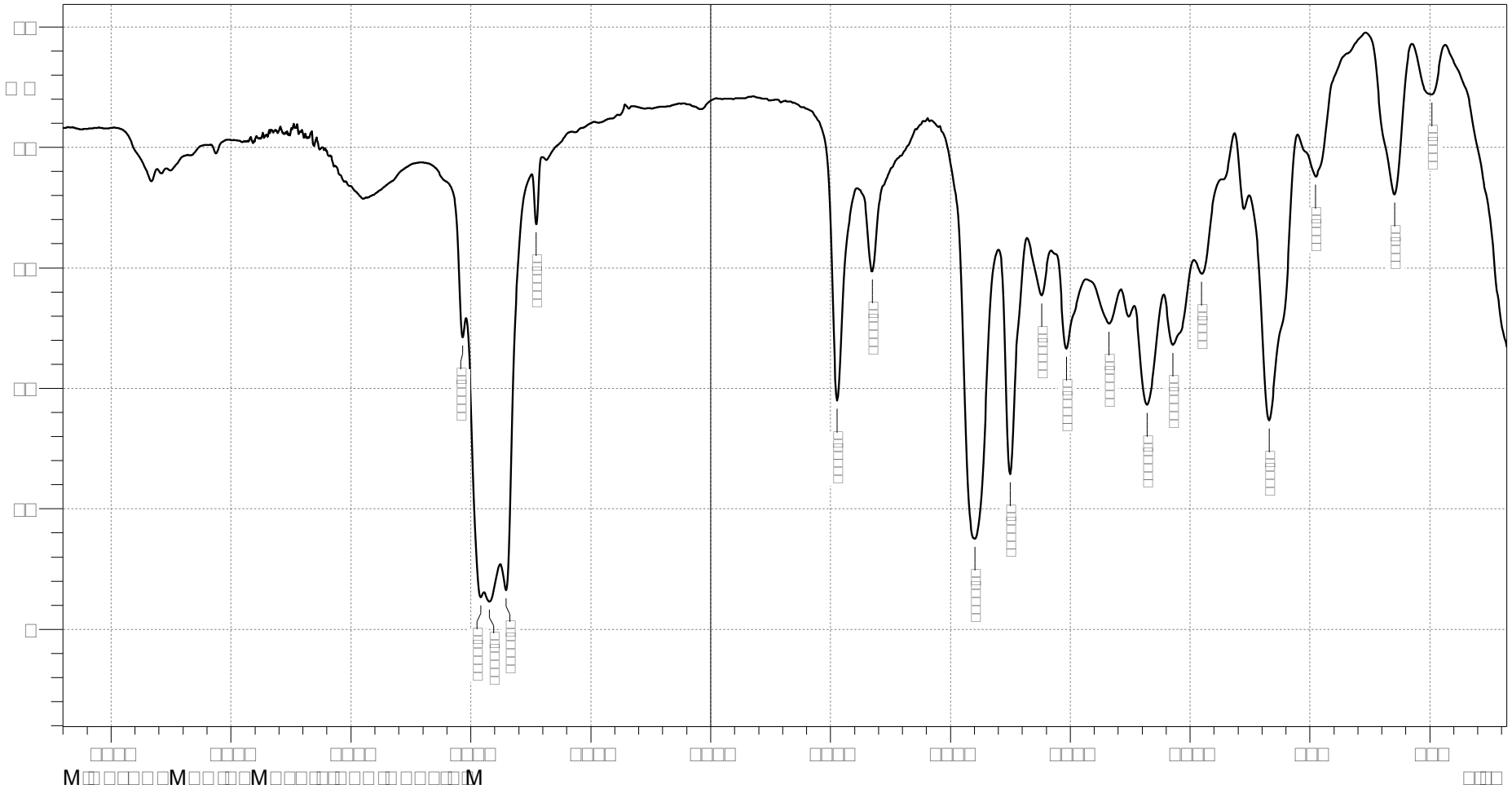


Comment;

Resolution;
Apodization;

Date/Time; 11/23/2011 12:30:41 PM
User; USIC

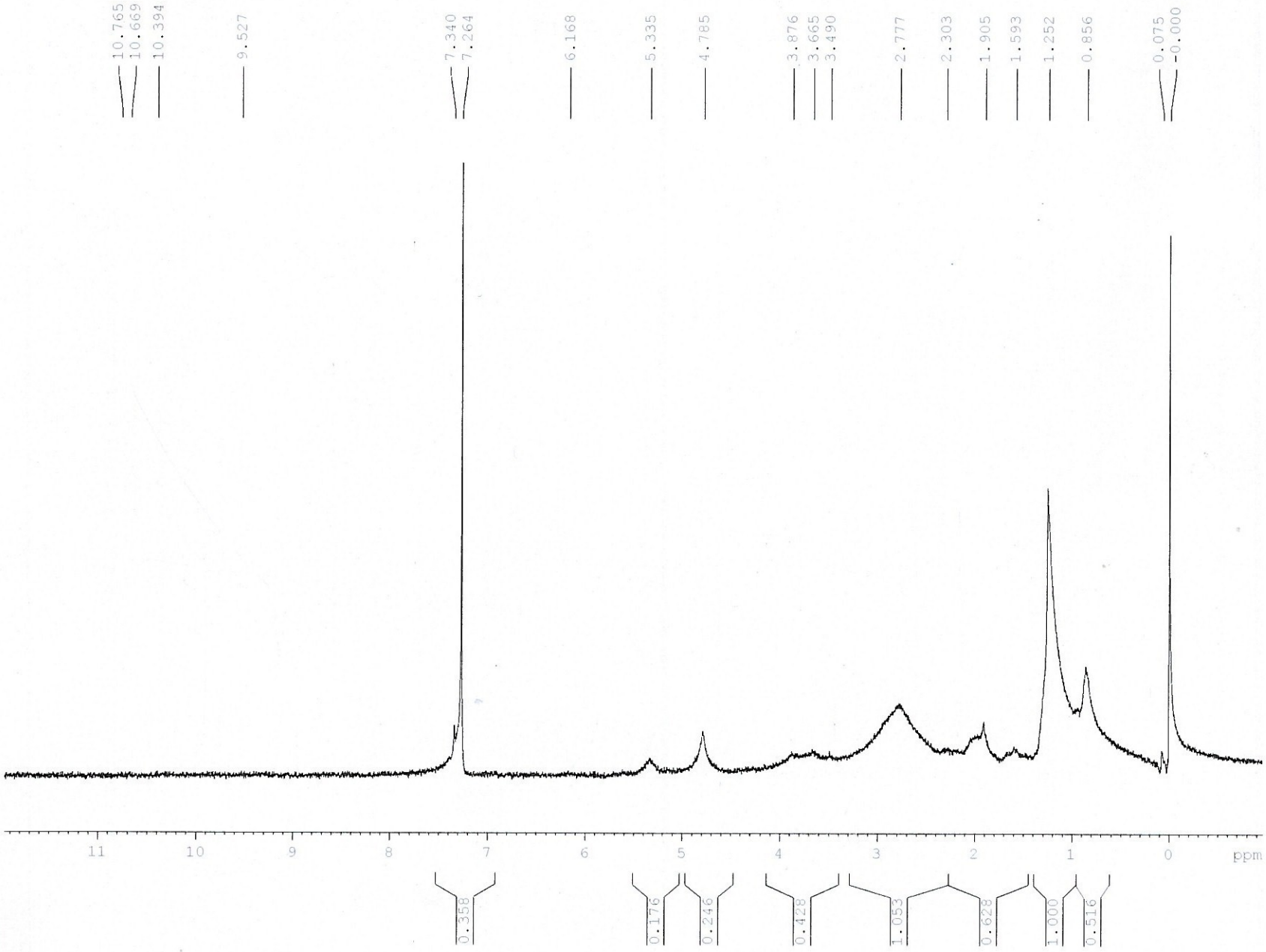
USIC-MKU



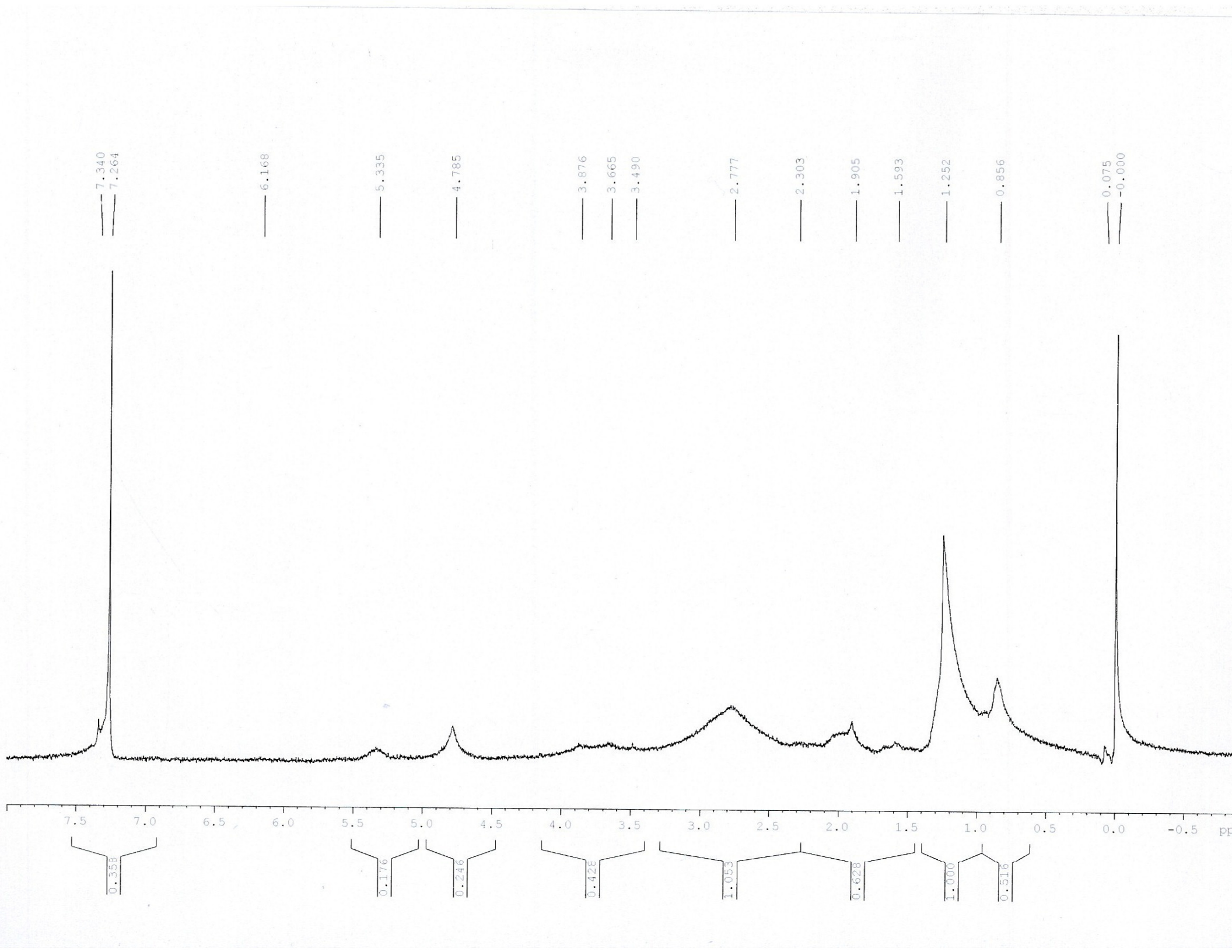
Comment;

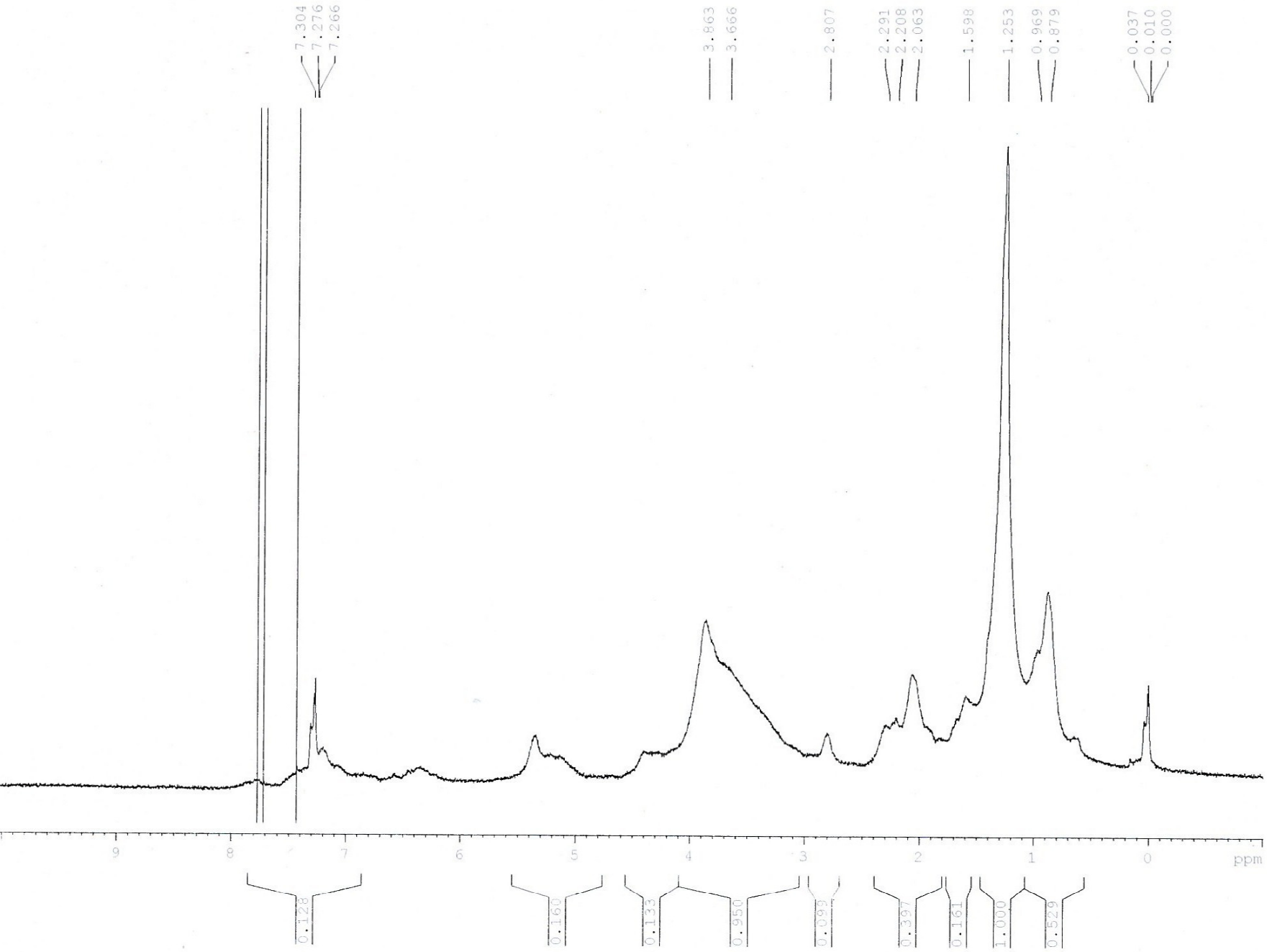
Resolution;
Apodization;

Date/Time; 11/23/2011 12:30:41 PM
User; USIC

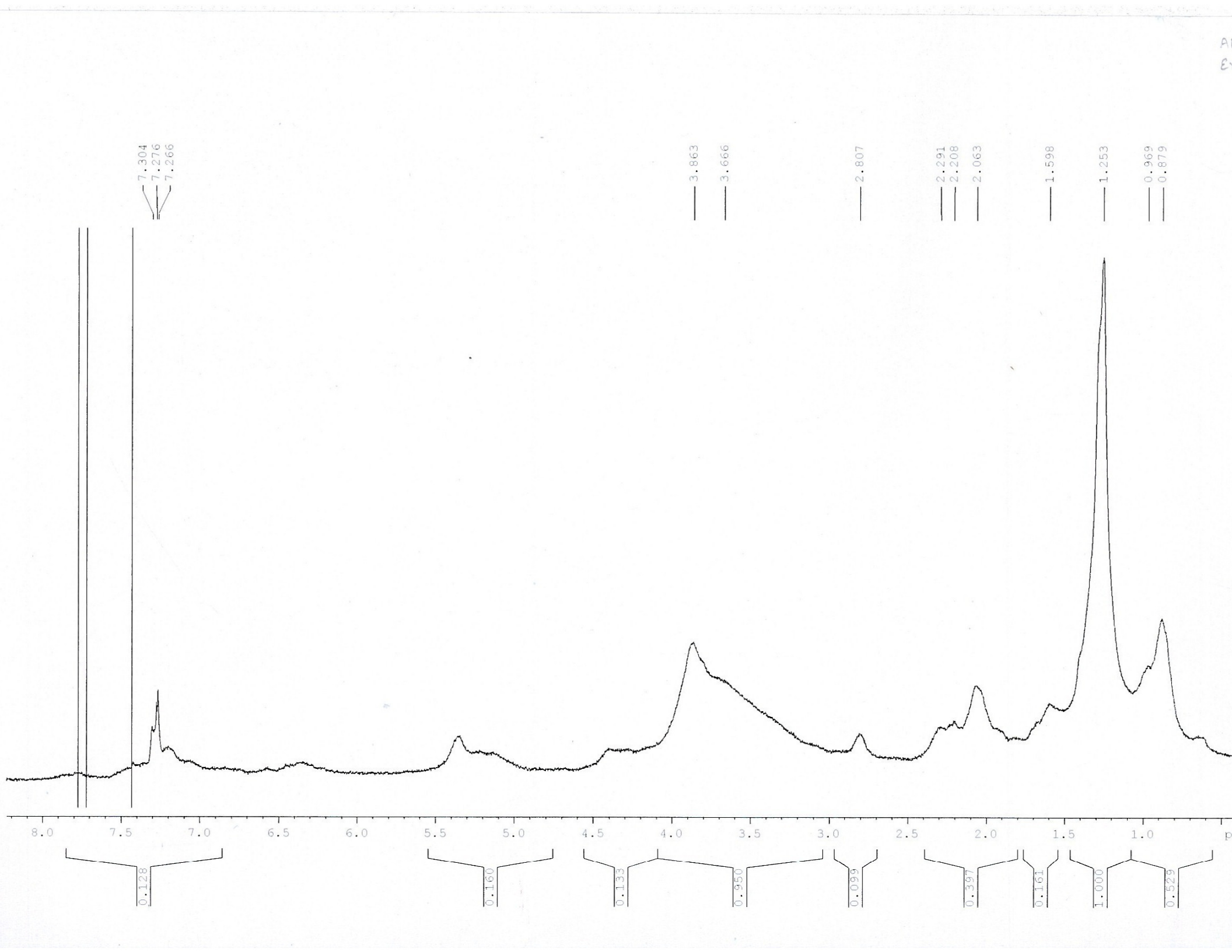


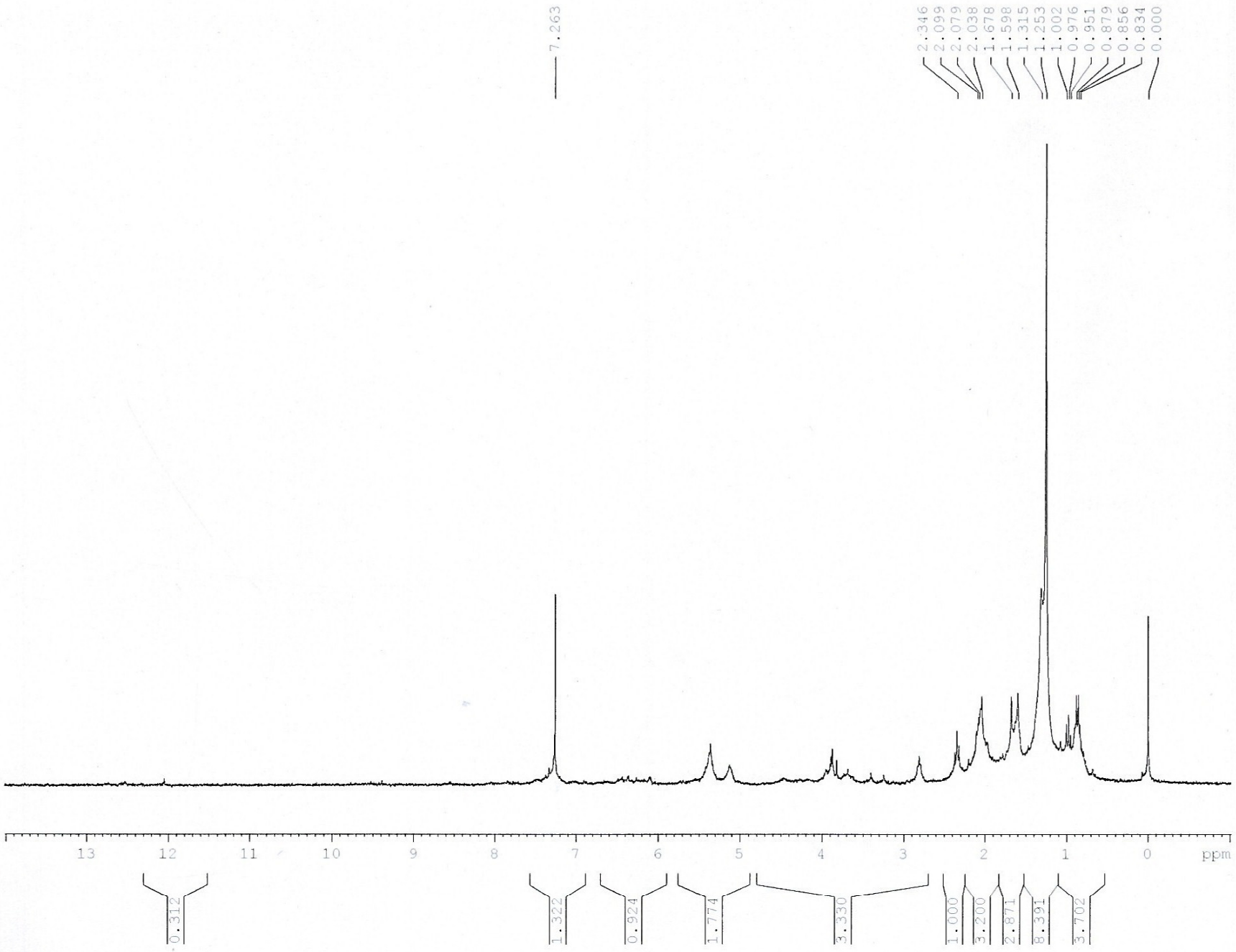

```
Current Data Parameters
NAME                  AEA
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_              20111117
Time                  15.08
INSTRUM               av300
PROBHD   5 mm BBO BB-1H
PULPROG               zg30
TD                   32768
SOLVENT              CDCl3
NS                      16
DS                       0
SWH               6172.839 Hz
FIDRES            0.188380 Hz
AQ                2.6542580 sec
RG                    161.3
DW                 81.000 usec
DE                  6.00 usec
TE                  300.0 K
D1              2.00000000 sec

======== CHANNEL f1 ========
NUC1                    1H
P1                   9.65 usec
PL1                  0.00 dB
SFO1          300.1318534 MHz

F2 - Processing parameters
SI                   32768
SF            300.1300064 MHz
WDW                     EM
SSB                      0
LB                   0.30 Hz
GB                       0
PC                    1.00
```


7.340
7.264
6.168
5.335
4.785
3.876
3.665
3.490
2.777
2.303
1.905
1.593
1.252
0.856
0.075
-0.000
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
0.358
0.176
0.246
0.428
1.053
0.628
1.000
0.516

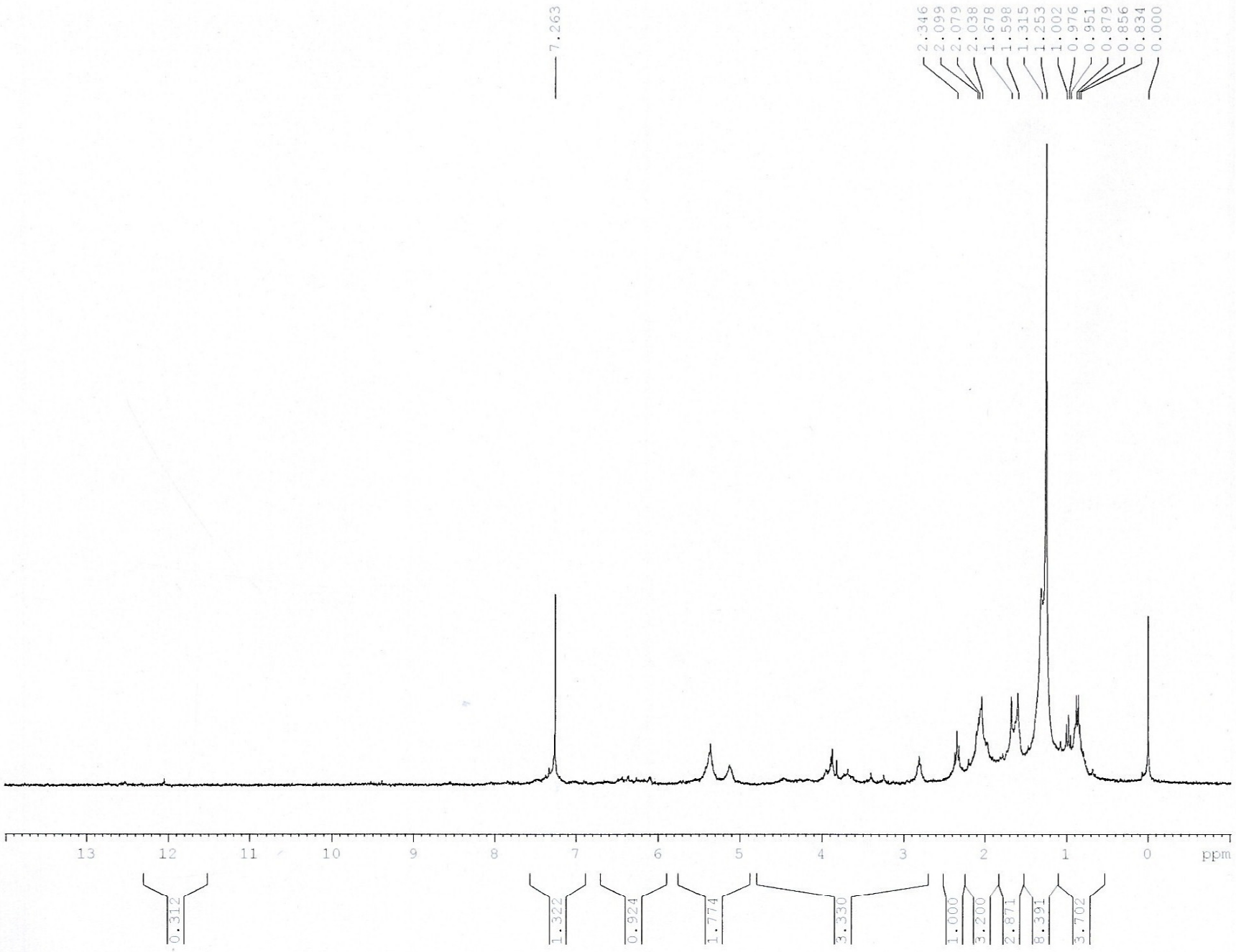



```
Current Data Parameters
NAME                  AEB
EXPNO                   1
PROCNO                  1

F2 - Acquisition Paramete
Date_              20111117
Time                  15.13
INSTRUM               av300
PROBHD   5 mm BBO BB-1H
PULPROG                zg30
TD                    32768
SOLVENT               CDCl3
NS                       16
DS                        0
SWH                6172.839 H
FIDRES                0.188380 H
AQ                    2.6542580 s
RG                      161.3
DW                     81.000 u
DE                      6.00 u
TE                     300.0 K
D1                 2.00000000 s

======== CHANNEL f1 =====
NUC1                      1H
P1                     9.65 u
PL1                    0.00 d
SFO1          300.1318534 M

F2 - Processing parameter
SI                    32768
SF            300.1300054 M
WDW                      EM
SSB                       0
LB                     0.30 H
GB                        0
PC                     1.00
```

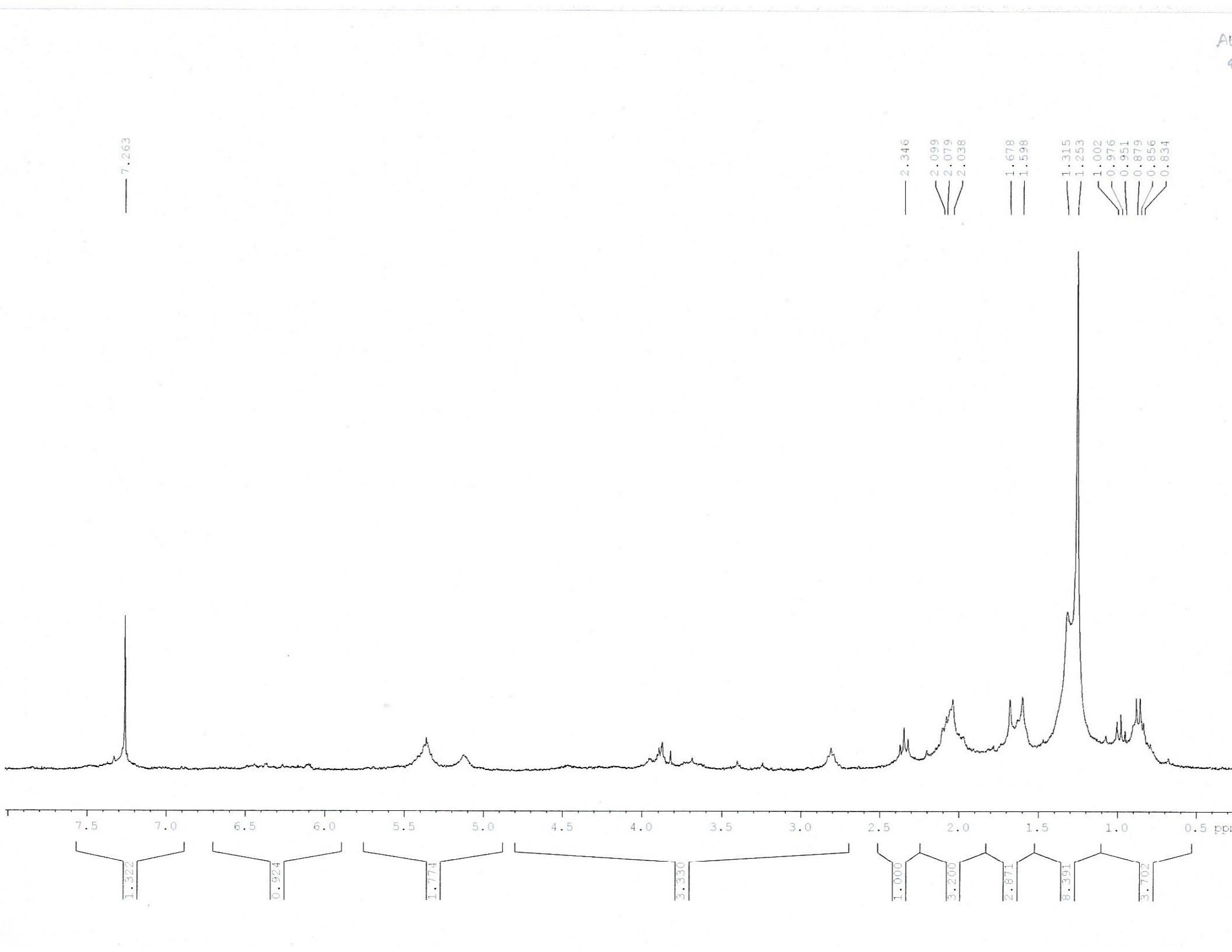

7.304
7.276
7.266
3.863
3.666
2.807
2.291
2.208
2.063
1.598
1.253
0.969
0.879
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.128
0.160
0.133
0.950
0.099
0.397
0.161
1.000
0.529

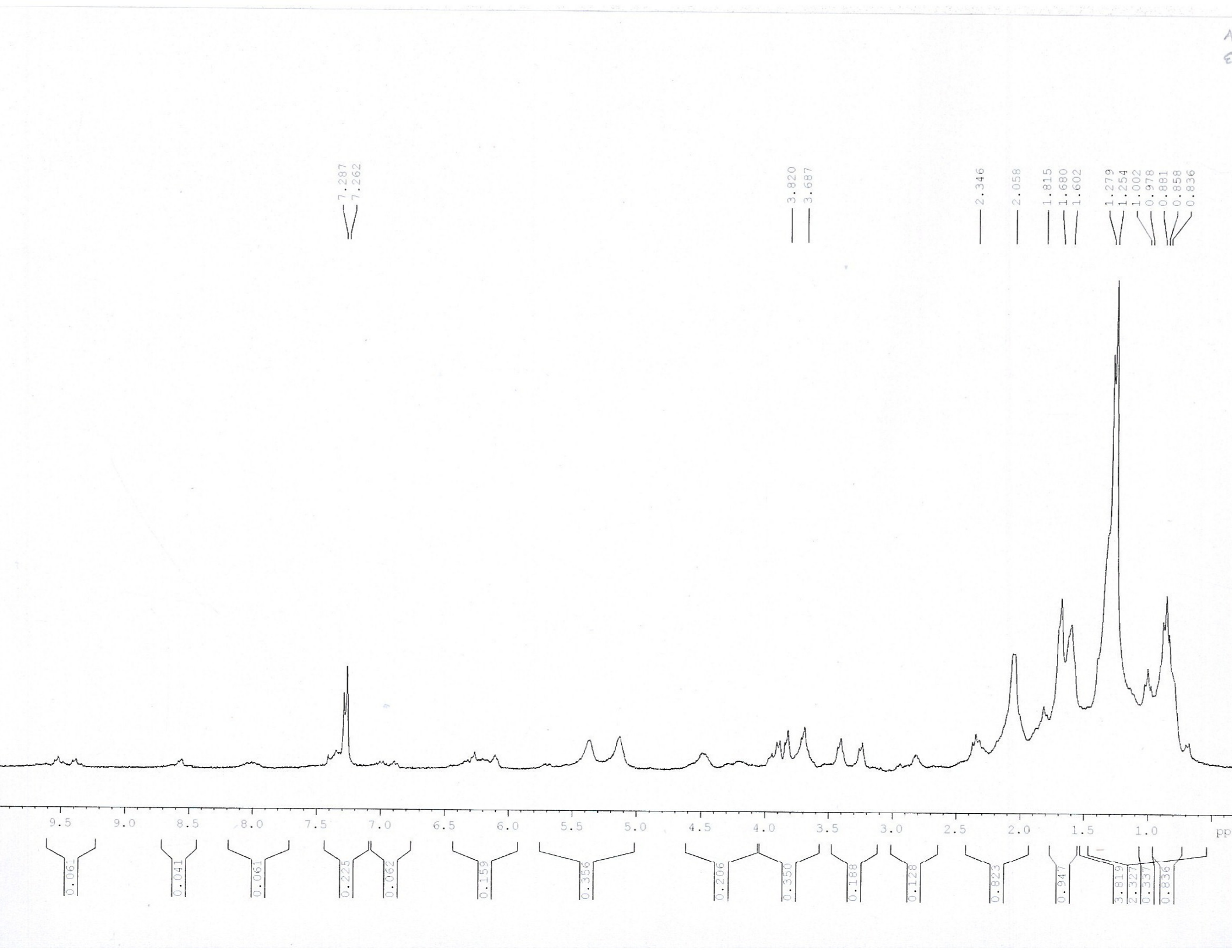


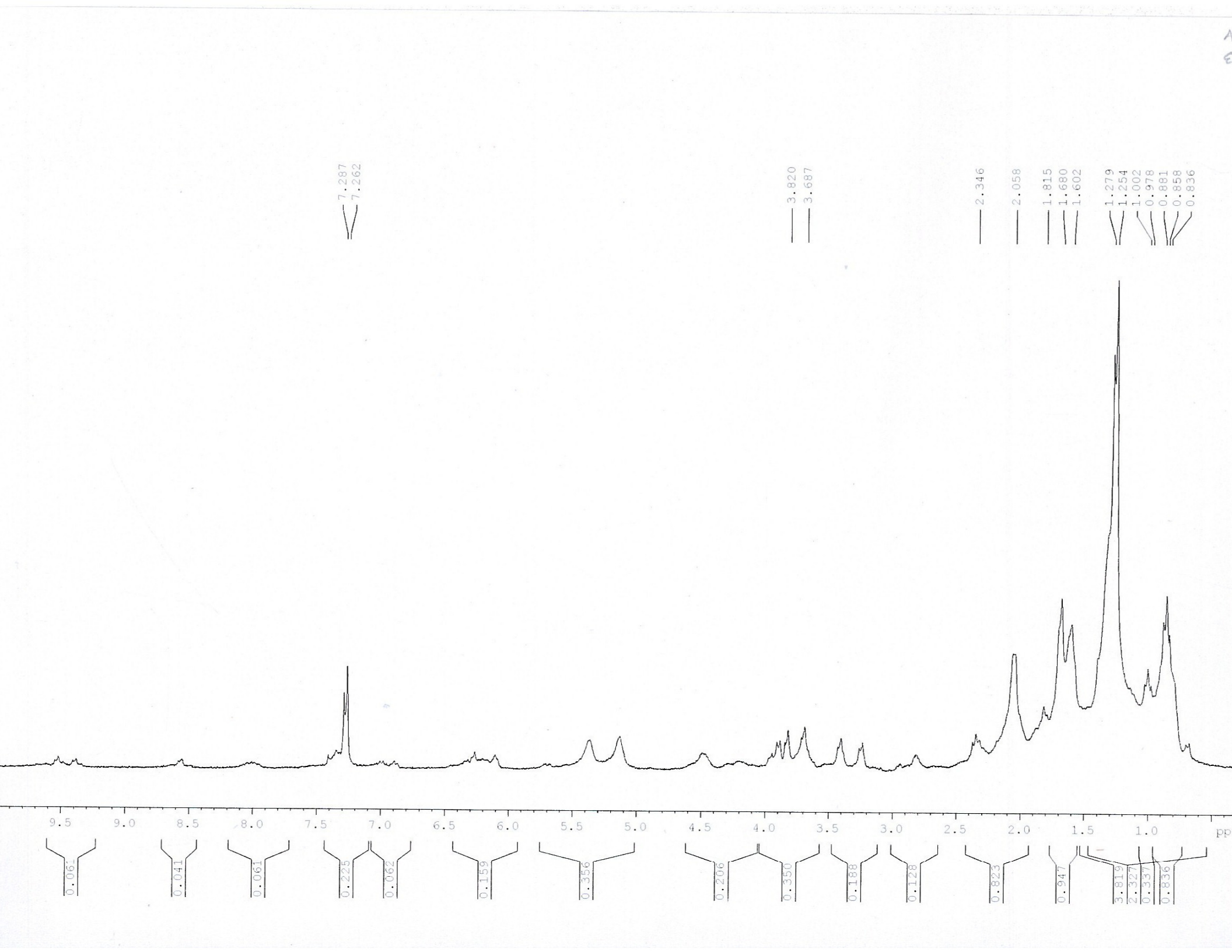

```
Current Data Parameters
NAME                      AEC
EXPNO                       1
PROCNO                      1

F2 - Acquisition Parameters
Date_              20111117
Time                  15.17
INSTRUM               av300
PROBHD    5 mm BBO BB-1H
PULPROG                zg30
TD                    32768
SOLVENT               CDCl3
NS                       16
DS                        0
SWH                6172.839 Hz
FIDRES             0.188380 Hz
AQ                 2.6542580 sec
RG                     161.3
DW                    81.000 use
DE                     6.00 use
TE                    300.0 K
D1                 2.00000000 sec

======== CHANNEL f1 ========
NUC1                      1H
P1                      9.65 use
PL1                     0.00 dB
SFO1           300.1318534 MHz

F2 - Processing parameters
SI                    32768
SF             300.1300065 MHz
```

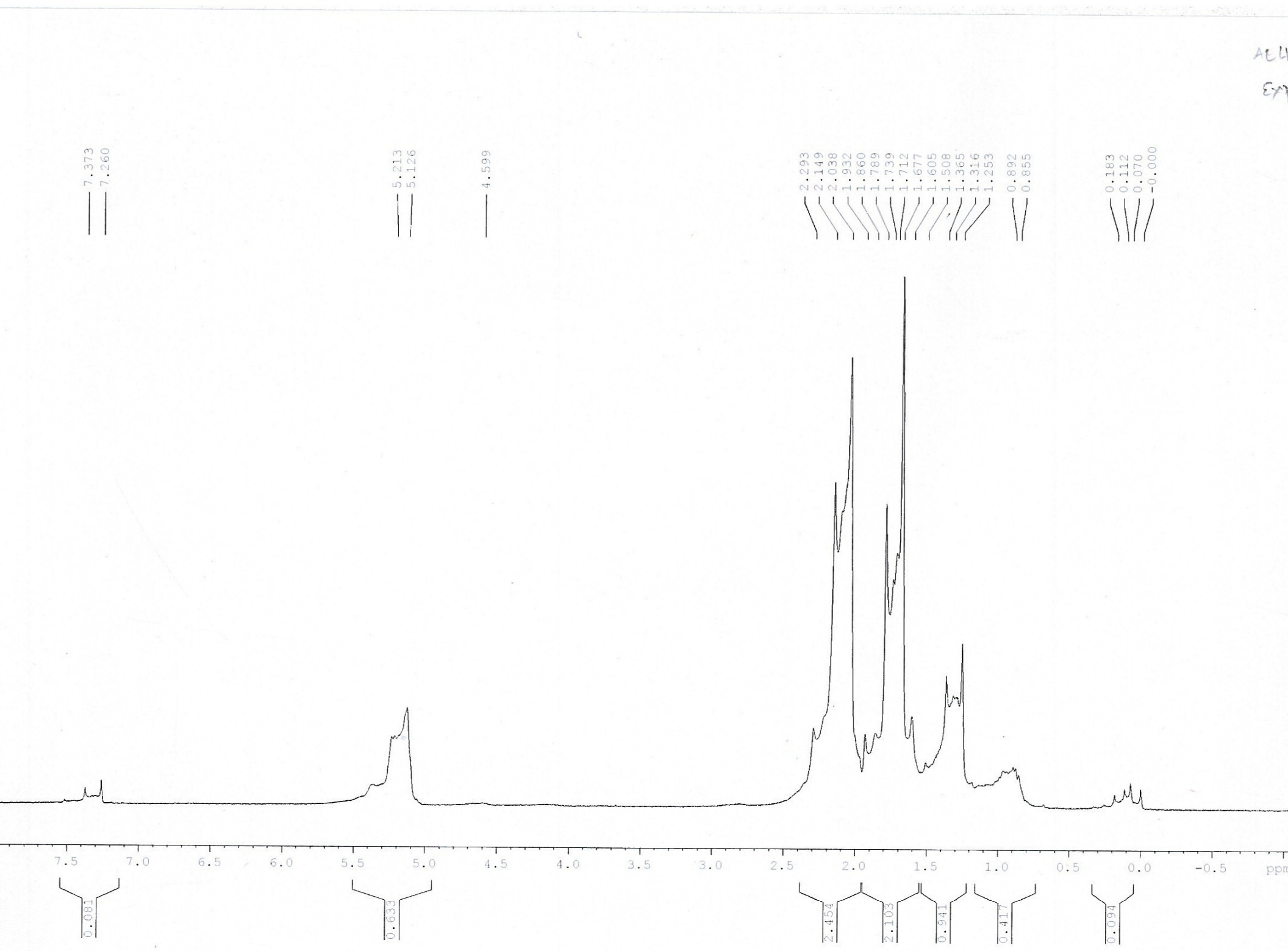


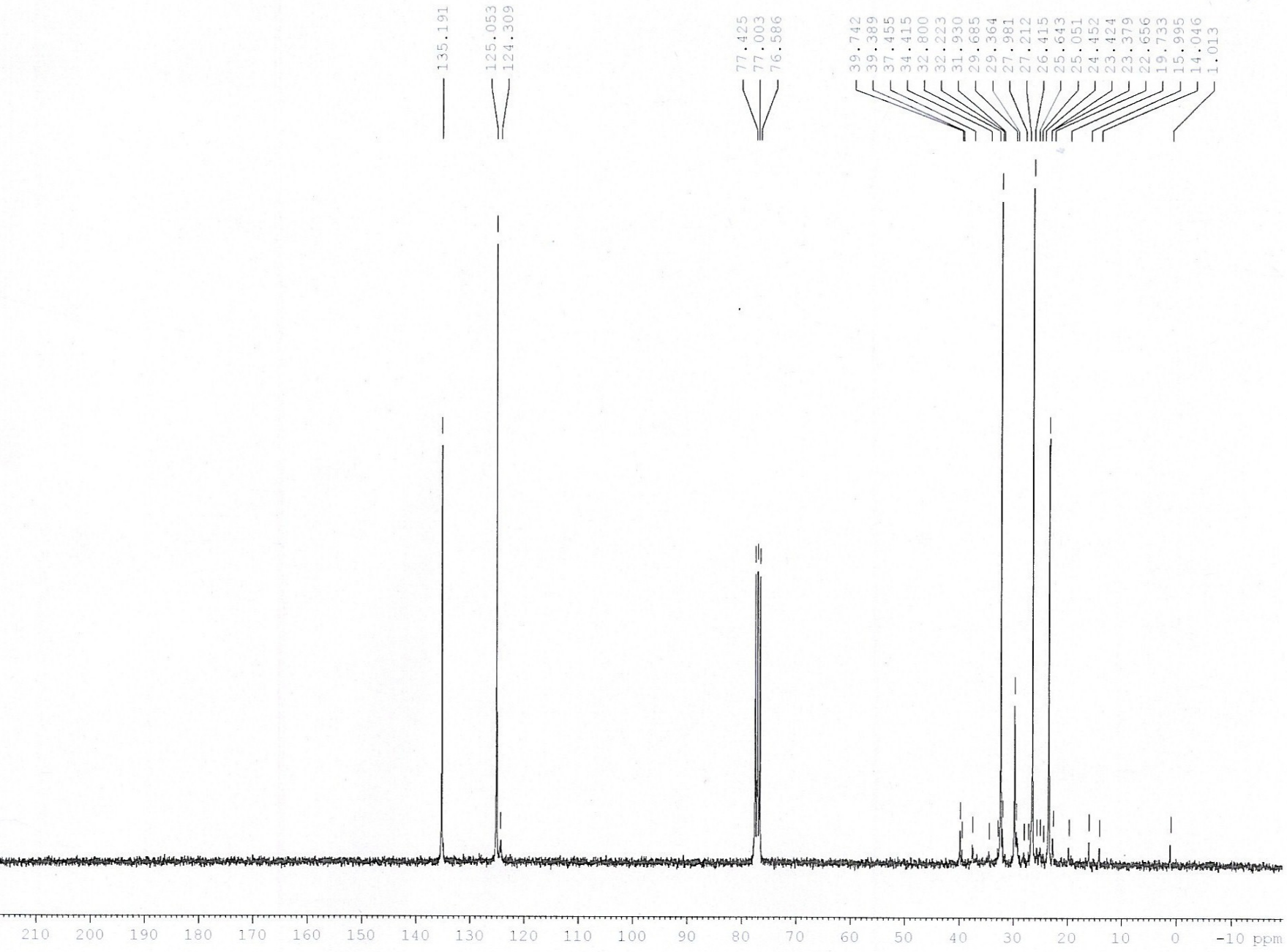

```
Current Data Parameters
NAME                      AEC
EXPNO                       1
PROCNO                      1

F2 - Acquisition Parameters
Date_              20111117
Time                  15.17
INSTRUM               av300
PROBHD    5 mm BBO BB-1H
PULPROG                zg30
TD                    32768
SOLVENT               CDCl3
NS                       16
DS                        0
SWH                6172.839 Hz
FIDRES             0.188380 Hz
AQ                 2.6542580 sec
RG                     161.3
DW                    81.000 use
DE                     6.00 use
TE                    300.0 K
D1                 2.00000000 sec

======== CHANNEL f1 ========
NUC1                      1H
P1                      9.65 use
PL1                     0.00 dB
SFO1           300.1318534 MHz

F2 - Processing parameters
SI                    32768
SF             300.1300065 MHz
```


7.263
2.346
2.099
2.079
2.038
1.678
1.598
1.315
1.253
1.002
0.976
0.951
0.879
0.856
0.834
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5 ppm
1.322
0.924
1.774
3.330
1.000
3.200
2.871
8.391
3.702


7.287
7.262
3.820
3.687
2.346
2.058
1.815
1.680
1.602
1.279
1.254
1.002
0.978
0.881
0.858
0.836
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
0.061
0.041
0.061
0.225
0.062
0.159
0.356
0.206
0.350
0.188
0.128
0.823
0.947
3.819
2.327
0.337
0.836


7.287
7.262
3.820
3.687
2.346
2.058
1.815
1.680
1.602
1.279
1.254
1.002
0.978
0.881
0.858
0.836
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
0.061
0.041
0.061
0.225
0.062
0.159
0.356
0.206
0.350
0.188
0.128
0.823
0.947
3.819
2.327
0.337
0.836


7.373
7.260
5.213
5.126
4.599
2.293
2.149
2.038
1.932
1.860
1.789
1.739
1.712
1.677
1.605
1.508
1.365
1.316
1.253
0.892
0.855
0.183
0.112
0.070
-0.000
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
ppm
0.081
0.633
2.454
2.103
0.941
0.417
0.094




```
Current Data Parameters
NAME                      AEH
EXPNO                       3
PROCNO                      1

F2 - Acquisition Paramete
Date_                20111125
Time                    11.28
INSTRUM                 av300
PROBHD    5 mm BBO BB-1H
PULPROG                zgpg30
TD                      65536
SOLVENT                 CDCl3
NS                       1370
DS                          4
SWH                 17985.611 H
FIDRES                0.274439 H
AQ                    1.8219508 s
RG                      14596.5
DW                       27.800 u
DE                        6.00 u
TE                       300.0 K
D1                    2.00000000 s
d11                   0.03000000 s
d12                   0.00002000 s

======== CHANNEL f1 =====
NUC1                       13C
P1                       12.50 u
PL1                      -5.00 d
SFO1                75.4752653 M

======== CHANNEL f2 =====
CPDPRG2                waltz16
NUC2                       1H
PCPD2                  100.00 u
PL2                      0.00 d
PL12                    20.31 d
PL13                    10.00 d
SFO2               300.1312005 M

F2 - Processing parameter
SI                       32768
SF                  75.4677495 M
WDW                         EM
SSB                          0
LB                        1.00 H
GB                           0
```

BIBLIOGRAPHY

1. C.S. Shah, J.S.Quardy. Introduction. In Text book of Pharmacognosy. Ahamedabad: B.S. Shah prakashan publishers; 1983. p. 1-15.

2. Trease G.E, Evance W.C. Introduction. In Text book of Pharmacognosy. London: W.B. Saunders; 2002. p. 1-8.

3. Varro E. Tyler, Lynn R. Brady, James E. Robbers. Natural products and phytochemistry. In Pharmacognosy. Philadelphia: Lea and Febiger; 1981. p. 6-13.

4. Mukherjee, Pulok K. Morphological evaluation. In Quality control of Herbal drugs. New Delhi: Business horizons pharmaceutical publishers; 1987. p. 3-15.

5. Khadewal. Leaves. In Practical Pharmacognosy. Pune: Nirali prakashan; 1988. p. 45-51.

6. Harborne, J.B. Methods of plant analysis. In Phytochemical methods. New Delhi: Springer; 1999. p. 1-32.

7. C.K. Kokate, A.P. Purohit, S.B. Gokhle. General Introduction. In Pharmacognosy. Pune: Nirali prakashan; 1997. p. 1-14.

8. Chatwal, Gurdeep. Terpenoids. In Organic chemistry of natural products. Mumbai: Himalaya publishing house; 2007. p.1.1-1.25

9. B. Jayaprakasam, A.G. Damua, D. Gunasekara, A.Blond, B. Bodo. Dihydroechioidinin, a flavone from Andrographis echioides. phytochemistry. 1999 may; 52: p. 935-937.

10. T.R. Govindhachari, P.C. Parthasarathy, B.R. Pai, P.S. Subramaniam. Chemical examination of Andrographis echioides-I Structure and synthesis of echioidinin. Tetrahedron. 1965; 21: p. 2633-2640.

11. T.R.Govindachari, P.C. Parthasarathy, B.R. Pai, P.S. Subramaniam. Chemical examination of Andrographis echioides-II Structure and synthesis of echioidin. Tetrahedron. 1965 july; 21: p. 3715-3720.

12. Y. Koteswara Rao, G.Vimalamma, C. Venkata Rao, Yew-Min Tzeng. Flavanoids and Andrographolides from Andrographis paniculata. Phytochemistry. 2004 Aug; 65: p. 2317-2321.

13. Poonam Kulyal, U.K. Tiwari, A. Shukla, A.K. Gaur. Chemical constituents isolated from Andrographis panaiculata. Indian Journal of Chemistry. 2010 March; 49B: p. 356-359.

14. Muntha K. Reddy, Mopuru V.B. Reddy, Duvvuru Gunasekar, Madugula M. Murthy, Cristelle Caux, Bernard Bodo. A flavone and an unusual 23-carbon terpenoid from Andrographis paniculata. Phytochemistry. 2003 Jan; 62: p. 1271-1275.

15. Guo-Cai Wang, Ying Wang, lan D Williams, Herman Ho-Yung Sung, Xiao-Qi Zhang, Dong-Mei Zhang, Ren-Wang Jiang, Xin-Sheng Yao, Wen-Cai Ye. Andrographolactone, a unique diterpene from Andrographis paniculata. Tetrahedron letters. 2009 may; 50: p. 4824-4826.

16. P. Hari Kishore, M. Vijaya Bhaskar Reddy, M. Kesava Reddy, D. Gunasekar, Cristelle Caux, Bernard Bodo. Flavanoids from Andrographis lineata. Phytochemistry. 2003 july; 63: p. 457-461.

17. Y. Koteswara Rao, P. Hari Kishore, C. Venkata Rao, D. Gunasekar, A. Blond, B.Bodo. Flavones from Andrographis viscosula. Phytochemistry. 2002 july; 61: p. 927-929.

18. P.K. Singh, Tanveer Hasan, Neeraj Misra. Vibrational dynamics of the Diterpene Neoandrographolide. E-journal of chemistry. 2009 Oct; 6(1): p. 183-188.

19. Lixia Chen, Huajie Zhu. ent-Labdane diterpenoid lactone stereoisomers from Andrographis paniculata. Journal of natural products. 2007 aug.

20. P. Premkumar, J. Priya, M. Suriyavathana. Evaluation of antioxidant potential of Andrographis echioides and Boehavia diffusa. International Journal of Current Research. 2010 April; 3: p. 059-062.

21. Radha, R. M. Sermakkani V. Thangapandian. Evaluation of preliminary phytochemical and antimicrobial activity of Andrographis echioides- L-Nees. Pharma science monito ran international journal of pharmaceutical science. 2011 Feb; 2(2): p. 091-101.

22. S.K. Basu, M. Rupeshkumar, K. Kavitha. Studies on the anti-inflammatory, analgesic and antipyretic properties of Andrographis echioides-L-Nees. International journal of pharmacology. 2009; 5(4): p. 251-256.

23. K. Kavitha, S.K. Basu, M.Rupeshkumar. Hepatoprotective and antioxidant effect of Andrographis echioides Nees against acetaminophen induced hepatotoxicity in rats. Journal biological science. 2009; 9(4): p. 351-356.

24. R. Radha, M. Sermakkani V. Thangapandian. Evaluation of phytochemical and antimicrobial activity of Andrographis paniculata nees (Acanthaceae) aerial parts. International Journal of pharmacy and life sciences. 2011 feb; 2(2): p. 562-567.

25. S.K. Ojha, M. Nandave, S. Kumari D.S. Arya. Antioxidant Activity of Andrographis paniculata in Ischemic Myocardium of Rats. Global Journal of Pharmacology. 2009; 3(3): p. 154-157.

26. Abubakar Sule, Qamar U. Ahmed, Othman A. Samah Muhammad N. Omar. Bacteriostatic and bactericidal activities of Andrographis paniculata extracts on skin disease causing pathogenic bacteria. Journal of Medicinal Plants Research. 2011 January; 5(1): p. 7-14.

27. S. Meenatchisundaram, G. Parameswari, A. Michael. Studies on antivenom activity of Andrographis paniculata and Aristolochia indica plant extracts against Daboia russelli venom by in vivo and in vitro methods. Indian Journal of Science and Technology. 2009 Mar; 2(4): p. 76-79.

28. Piengpen Thisoda, Nuchanart Rangkadilok, Nanthanit Pholphana, Luksamee Worasuttayangkurn, Somsak Ruchirawat, Jutamaad Satayavivad. Inhibitory effect of Andrographis paniculata extract and its active diterpenoids on platelet aggregation. European Journal of Pharmacology. 2006 march; 559: p. 039-045.

29. Rammohan Subramanian, Mohd. Zaini Asmawi, Amirin Sadikun. Effect of ethanolic extract of andrographis paniculata (burm. f.) nees on a combination of fat-fed diet and low dose streptozotocin induced chronic insulin resistance in rats. Diabetologia Croatica. 2008 Feb; 37(1): p. 013-022.

30. Vetriselvan.S, Victor rajamanickam, Muthappan.M, Gnanasekaran.D, Dinesh kumar chellapan. Hepatoprotective effect of aqueous extract of Andrographis paniculata against CCl4 induced hepatotoxicity in albino wistar rats. Asian Journal of Pharmaceutical and Clinical Research. 2011; 4(3): p. 93-94.

31. Sutha, Devaraj, Jegathambigai R, Kumar P, Sudhakaran Sivaramakrishnan. A study on the hepatopretective effect of Andrographis paniculata (burm.f) Nees on mice. Journal of Phytology Phytopharmacology. 2010; 2(11): p. 25-30.

32. Kanokwan Jarukamjorn, Nobuo Nemoto. Pharmacological aspects of Andrographis paniculata on health and its major diterpenoid constituent Andrographolide. Journal of health sciene. 2008; 54(4): p. 370-381

33. K. Sheeja, C. Guruvayoorappan, G. Kuttan. Antiangiogenic activity of Andrographis paniculata extract and andrographolide. International Immunopharmacology. 2007 Aug; 7: p. 211–221.

34. R. Ajaya Kumar, K. Sridevi, N. Vijaya Kumar, S. Nanduri, S. Rajagopal. Anticancer and immunostimulatory compounds from Andrographis paniculata. Journal of Ethnopharmacology. 2004 Feb; 92: p. 291-295.

35. E. Arnroyan, E. Cabrielian, A. Panossiarr, G. Wikman, H. Wagner. Inhibitory effect of andrographolide from Andrographis paniculata on PAF-induced platelet aggregation. Phytomedicine. 1999; 6(1): p. 027-031.

36. Reddy VL, Reddy SM, Ravikanth V, Krishnaiah P, Goud TV. A new bis-andrographolide ether from Andrographis paniculata Nees and evaluation of anti-HIV activity. Natural product res. 2005 April; 19(3): p. 223-230.

37. Wiart C, Kumar K, Yusof MY, Hamimah H, Fauzi ZM, Sulaiman M. Antiviral properties of ent-labdane diterpenes of Andrographis paniculata nees , inhibitor of herpes simplex virus type 1. Phytother. 2005 Dec; 19(12): p. 1069-1070.

38. Singha PK, Roy S, Dey S. Antimicrobial activity of Andrographis paniculata. 2003 Dec; 74(7-8): p. 692-694.

39. Zhang CY, Tan BK. Hypotensive activity of aqueous extract of Andrographis paniculata in rats. Clin Exp Pharmacol Physiol. 1996 Aug; 23(8): p. 675-678.

40. Borhanuddin M, Shamsuzzoha M, Hussain AH. Hypoglycaemic effects of Andrographis paniculata Nees on non-diabetic rabbits. Bangladesh Med Res Counc Bull. 1994 Apr; 20(1): p. 24-26.

41. Rajagopal S, Kumar RA, Deevi DS, Satyanarayana C, Rajagopalan R. Andrographolide, a potential cancer therapeutic agent isolated fromAndrographis paniculata. J Exp Ther Oncol. 2003 Jun; 3(3): p. 147-58

42. Akhtar Hussain, O.P. Virmani, S.P. Popli. Andrographis echioides. In Dictionary of Indian medicinal plants. Lucknow: Central institute of medicinal aromatic plants; 1992. p. 32-33.

43. Chadha, Y.R. Andrographis. In The wealth of India. Newdelhi: Publication and Information directorate CSIR; 1885. p. 264-268.

44. K.R. Kirtikar, B.D. Basu. Andrographis echioides. In Indian medicinal plants. Dehradun: International book distributors; 1987. p. 1886.

45. R.N. Chopra, S.L. Nayar, I.C. Chopra. Andrographis echioides. In Glossary of Indian medicinal plants. Newdelhi: council of scientific and industrial research; 1980. p. 18.

46. V. Satyavati, M.K. Raina, M. Sharma. Andrographis. In Medicinal plants of India. Newdelhi: Indian council of medical research; 1976. p. 64-67

47. A.H.Beckett, J.B. Stanlake. Chramatography. In Practical Pharmaceutical Chemistry. Newdelhi: CBS publishers and distributors; 2005. p. 85-174.

48. Bobbit, James M. Thin layer chromatography; Mumbai: Reinhold publishing corporation; 1963. p. 1-11

49. Hilde bert Wagner, Sabine Bladt. Thin layer chromatography. In Plant drug analysis.; 1995. p. 125.

50. Douglas A. Skoog, F.James, Holler, Nieman. Infrared spectrometry. In Principles of Instrumental Analysis. Australia: Thomson brooks/cole; 2003. p. 381-428.

51. Elbs, William Kemp. IR and NMR. In Organic spectroscopy.; 1979.

52. K. Peach, M.V. Treacay. NMR spectroscopy. In Modern methods of plant analysis.; 2001. p. 462-474.

53. Kaur, H. NMR. In Spectroscopy. Meerut: Pragati prakashan; 2010. p. 322-414.

54. R.M. Silverstein, G. Clayton Bassler, Terence.C. Mossill. NMR Spectroscopy. In Spectrometric identification of organic compounds. New York: John Wiley and sons. INC; 2000. p. 91-226.

55. Sharma, Y.R. Infrared and NMR. In Elementary organic spectroscopy. Newdelhi: S.Chand and company ltd; 2004. p. 69-155, 182-255.

56. Edwin K Jackson, Goodman, Gilman. Diurtics. In The Pharmacological basics of therapeutics. New York: McGraw-Hill medical publishing division; 2005. p. 737-770.

57. Iswariah V, Guruswami M N. Diuretics. In Pharmacology and Pharmacotherapeutics.; 1979. p. 422-424.

58. Parimoo, P. Classification of diuretics. In Text book of Medicinal chemistry.; 1988. p. 67-77.

59. K.D. Tripathi. Diuretics. In Essential of pharmacology. Newdelhi: Jaypee brothers medical publishers pvt ltd; 2003. p. 234-245.